VOLATILE FLAVOR COMPONENTS OF CELERY STALKS (*Apium graveolens* VAR. *dulce*) AS RELATED TO TEMPERATURE AND TIME IN STORAGE– WITH FURTHER INVESTIGATIONS ON COMPONENT DISTRIBUTION WITHIN THE STALK

By
DANNY ODELL EZELL

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INTRODUCTION

With the increasing sophistication in analytical techniques and instrumentation has come a greater knowledge on the volatile constituents of foods. Although the flavor of celery (Apium graveolens var. dulce) is quite distinctive, it has received relatively little attention as compared to other fruit and vegetable flavors.

Vegetables are in many cases quite difficult to examine chemically. Flavor substances frequently occur in concentrations of a few parts per million, while accompanied by other organic materials and large amounts of water. Several extraction (38) and analytical (39) techniques have allowed increased resolution of these flavor substances and a decrease in interference from foreign materials.

Celery is a perishable agricultural commodity; however, with proper handling it can be maintained in a marketable condition for moderate periods of time. The long storage durations involved in shipping Florida celery to Europe have imposed more stringent demands on the keeping quality of fresh celery. Quality reduction of celery due to storage duration is usually manifest by water loss, pithiness, loss of green color, decrease in sucrose and increasing toughness. While flavor changes do exist as a result of

storage, it has not been determined if these flavor changes are caused by changes in the volatile flavor constituents or if these changes result in a detriment to quality.

This research was initiated to: i. select a procedure for extraction and measurement of the volatile flavor components in celery stalks; ii. observe whether differences occur in the chromatograms of celery extracts as a result of storage temperature and duration; iii. observe differences in the chromatograms of head-space volatiles (vapor pressures sufficient to permit analysis in the vapor phase without extraction) obtained from celery as a result of storage temperature and duration, iv. investigate the flavor profile of the various portions of the stalk of celery.

REVIEW OF LITERATURE

The maintenance of quality of fresh celery in storage involves several aspects of applied physiology. Corbett (8) indicated that several physical and chemical changes were apparent during the storage of celery. Chemical analyses of different parts of the plant showed marked changes in reducing and total sugars and also both soluble and insoluble nitrogen. Reducing sugars decreased in the leaves from harvest to the end of storage. Young (45) and White-Stephens (43) also concluded there was an increase in dry weight in the crown portion of the celery plant with a corresponding decrease in the outer leaf portion, and the outer leaves of the pascal celery plant were of definite value in growth of the heart petioles during storage. White-Stephens also observed a marked increase in sucrose and polysaccharides in the inner petioles coincident with a similar decrease in the outer petioles. Corbett (8) concluded that soluble nitrogen increased in both leaves and stalks from harvest to near the end of the storage period, at which time there was a very marked decrease in the leaves. This decrease of soluble nitrogen in the leaves resulted in an increase in the inner petioles.

Hall, et al. (24) cautioned against making quality

comparisons between different varieties of celery at different maturities. Single varieties of celery seemed to follow the same pattern of change in chlorophyll content, alcohol insoluble solids, total sugars, crude fiber, and dry weight with regard to increasing maturity. Three varieties followed the same general trend, but varied in the time the lowest and highest points were reached.

Organoleptic (taste) comparisons have been made between celery (Utah 52-70 variety) grown in Florida and the same type celery produced in California. When considering celery from an April harvest, the taste panel indicated the Florida celery had a superior flavor, but was tougher and had more fiber. However, when comparisons were made with celery from a May harvest, the rankings indicated that the California celery had a more desirable flavor and was less bitter, but was tougher and more fibrous than the Florida-grown celery. In these experiments it was noted that a low potassium-sodium ratio was associated with a bland flavor. The celery from the two areas was grown by commerical producers, was the same age, and was handled under similar conditions.

The price of Florida celery has frequently been below that of celery produced in other areas. While these opinions were not unanimous, the reasons given for the reduced price included poor appearance, bitter flavor and toughness (22).

While making observations on the flavor of celery, Hall (23) described a bitter fraction and a burning-numbing

sensation from celery. The bulk of the bitter flavor was associated with the dark green outer layer of the petiole and did not appear to be associated with the burning-numbing sensation. Description of the various flavors were: salty flavor, radish-like flavor, and a hydrocarbon-type flavor described as kerosene-or turpentine-like. The panel occasionally noted a sweet flavor. Hall also found a considerable difference in the composition and organoleptic ratings of outer, inner, and heart petioles of celery (21). The composition of these petioles was influenced by the temperature at which the celery was stored. In addition to the differences found between petioles according to position, there were differences in flavor and composition between middle and upper portions of the outer, inner, and heart petioles.

Using chromatographic analyses, Gold and Wilson (14) showed that not all flavor constituents were in the juice of celery stalks. The chromatograms prepared from juice and puree showed common peaks, although the chromatograms were not identical. An organoleptic study was performed on the juice of celery from the top (leafy) and basal portion of the celery stalk. The taste panel was able to differentiate between the juice from the top (leafy) and basal portions of stalks at the 0.01 level of significance. However, Gold and Wilson were not able to differentiate between the chromatograms prepared from the two juices. There was no difference between chromatograms prepared from fresh and frozen juice samples.

Pan (36) characterized the burning-numbing taste of celery using gas-liquid chromatography. This chemical had no unsaturated bonds or nitro groups. He found the presence of aldehydes, carbonyl, phenolic, hydroxy, and aromatic groups but the fraction was not a lactone. In further research (37), Pan described a new technique in the isolation of a bitter principle from celery. This principle was cationic at pH greater than 7, soluble in polar solvents, and fluorescent under ultraviolet light. The bitter principle could not be steam distilled from celery and was located in the dark green portion of the petiole.

The complex mixture which constitutes the flavor and odor of celery received considerable attention as early as 1897. Ciamician and Silber (7) reported the following terpenes contributing to the flavor of celery: limonene, myrcene, and an isomer of apiol. Of the lactone fractions identified, sedanolide and sedanonic anhydride were proposed as being of primary importance in the odor of celery seed. Following the identification of sedanolide and sedanonic anhydride by Ciamician and Silber, Berlingozzi and Cione (4) undertook a study of the chemistry and odor characteristics of alkyl and alkylidene phthalides. Working with $\Delta^{2,6}$-dihydrophthalide, Δ^{6}-tetrahydrophthalides, and hexahydrophthalides, they found when one of the γ-carbon hydrogens was replaced by an alkyl group, a celery odor was noted. When both were replaced by alkyl groups, the odor was less intense. Celery odor was most intense when

the γ-carbon hydrogen was replaced by the alkylidene group. Intensity increased as carbons increased from 1 to 4.

Barton and DeVaries (2) analyzed celery oil and reported the isolation of butyl phthalide instead of sedanolide when using Ciamician's method (7). They assumed that an unstable sedanolide might be changed to butyl phthalide, and proposed the structure of an α, β-unsaturated lactone, neocnidilide. Mitsuhashi and Muramatsu (33) further proposed that sedanolide is at least a mixture of neocnidilide and butyl phthalide.

Recent investigations concerning the flavor and odor of celery were conducted by Gold and Wilson (14, 15, 16) and Wilson (44). In 1963 (16), they listed 38 compounds which were identified as volatile components of celery. While most of the compounds listed probably make some contribution to the composite flavor and aroma of celery, 6 are of primary importance: 3-iso butylidene-and 3-iso validene-3a, 4-dihydrophthalide; 3-isobutylidene-and 3-isovalidene-phthalide; cis-3-hexen-1-yl pyruvate; and diacetyl. The 4 phthalide derivitives were found in a ratio of 6:3:1:1.

The difficulty of separating the elements of a chemical mixture into their respective flavor potencies has been pointed out (17, 40). Guadagni, et al. (18) found an additive effect of various chemicals even when the chemicals were present in sub-threshold concentrations. This difficulty was further emphasized in attempts to measure sensory responses for food products by direct injection

of aqueous vapors into a gas chromatograph (6, 29, 35). Newar (35) noted that the concentration of a given compound in a vapor phase at a given temperature is affected by: vapor pressure of the compound, type of media in which it is distributed, degree of solubility in the media, concentration of compound in liquid phase, and its miscibility with other organic compounds. Therefore, a decrease in liquid concentration does not necessarily mean a decrease in vapor concentration.

While there has been much research concerned with the quality of celery in storage and with the volatile flavor components of celery, little research has been conducted to investigate changes in these volatile components during storage, and it is to this question that the following work is addressed.

MATERIALS AND METHODS

Florimart and Utah 52-70-2-13 (commonly known as Florida-2-13) cultivars of celery were obtained from commercial growers. Size Number 3 stalks (36/crate) were harvested 90 days after transplanting, packed, hydrocooled, and transported immediately to the laboratory. Preliminary investigations were conducted to determine the most satisfactory method for extraction of celery volatiles and measurement of the various parameters.

Storage

Phase I

The initial study was established to determine the effect of low and high temperature storage on the composition of flavor extracts prepared from celery stalks. Low temperature effects were determined by comparing extracts from freshly harvested Florimart celery with those from celery stored 2 or 4 weeks at 38°F. High temperature effects were determined by comparing extracts from freshly harvested celery with those from celery stored 5 days at 70°F. A randomized block design was used with 3 harvest dates constituting blocks. A non-replicated study involved weekly extracts of celery stored at 38°F for 10 weeks.

Phase II

Florimart cultivar was also used in a storage treatment to simulate market conditions. Treatment involved placing the stalks at 45°F for 2 weeks and transferring samples to 50°F for an additional week. The experiment was a randomized block with 2 harvest dates as blocks and a duplicate analysis of each sample.

Phase III

Florida 2-13 cultivar was used for aroma (head-space) measurements. Treatments employed for these analyses are listed in Table 1. Statistical design of the experiment was a randomized block with 3 replications and triplicate analyses of each sample; harvest dates constituted blocks. Dry weights were determined according to conventional procedures.

Component Distribution

Celery stalks of the Florimart cultivar were divided vertically into top (leafy), middle, and bottom and, horizontally into outer and inner portions. Extracts were prepared of each sample to derive further information on the distribution of the volatile components of the vapor profile within the plant. Three replications were used with a completely randomized design.

Analytical Procedures

Organoleptic

A triangular test was used for all organoleptic

Table 1. Storage treatments used in Phase III.

Treatment	Storage Temperature	Duration	Subsequent Storage
	(°F)	(Weeks)	
0	At harvest	--	--
1	38	1	--
2	38	2	--
3	38	3	--
4	38	4	--
5	38	2	1 day at 70°
6	38	4	1 day at 70°
7	38	2	8 days at 50°
8	38	4	8 days at 50°
9	45	2	--
10	45	4	--

measurements (38). Organoleptic comparisons were made between freshly harvested celery and celery stored 2 weeks at 38°F. All organoleptic tests were conducted with a consumer-type panel composed of staff personnel. Panel scores were obtained in a taste panel room with subdued lighting. Samples were taken by dicing the center one-third of each inner petiole, excluding all heart petioles, from 20 stalks. Petioles with great visual differences were excluded.

Extraction

Celery petioles were washed and stripped of leaves. All petioles were ground in a Waring blendor and the juice expressed by hand. Each extraction sample consisted of 4,000 ml of juice which was taken from approximately 12 lb. of petioles. The juice was introduced in 400 ml portions into the evaporation chamber of a Nester-Faust model 500 rotary spray evaporator. The residue was removed from the evaporation chamber before each new aliquot of juice was introduced. The evaporation chamber was immersed in a water bath at 70°C and maintained at a pressure of 30 mm of mercury, while the condensor and collection chamber were maintained at 0°C and 30 mm of mercury. Preliminary procedures differed in that traps for collection of the flavor components were at temperatures of dry ice-acetone and liquid nitrogen and were placed after the cold water condensor (14, 16).

The clear aqueous condensate containing the volatile flavor constituents was retained for solvent extraction.

Dichloromethane, amounting to approximately 10 per cent of the volume of essence, was shaken with the essence of 2 minutes. The samples were washed 2 times and dried with sodium sulfate according to conventional methods. Solvent was partially removed in a rotary evaporator at 104°F with a reduced pressure of 660 mm of mercury. The residue was removed from the rotary evaporator when approximately 10 ml remained in the flask. Further removal of the solvent was accomplished at room temperature and atmospheric pressure.

Head-space samples were prepared by placing 500 grams of diced celery into a 1,000 ml erlenmeyer flask. The flasks were sealed and placed in a water bath at 55°C for 2 hours. At the end of this period, the head-space gas was taken by syringe for direct injection into the chromatograph. Standards were prepared by placing known quantities of the various chemicals on tissue paper and positioning in the center of the flask's contents.

Chromatographic

All chromatograms were prepared on an Aerograph Model 600 D chromatograph equipped with a flame ionization detector. Injection sample size and instrument parameters were maintained constant for each experiment. The sample volumes were 0.5 ul and 2.5 ml for solvent residue and head-space samples, respectively. The injection port was maintained at 235°C. Helium was used as a carrier gas with an inlet pressure of 40 psi and flow rate of 20 ml/min at room

temperature. A manual matrix programmed sequence was used from 100°C to 240°C at 3°/min as shown in Table 2. Hydrogen flow was 20 ml/min while the air was maintained at 250 ml/min. Chart speed was ½ in/min.

Treatment comparisons were made on 12' x 1/8" copper columns packed with 5 per cent W/W Apiezon L and Carbowax 20 M, both supported on 80/100 mesh Chromasorb G.

Subtractive chromatography for alcohols and aldehydes was performed by procedures described by Ikeda, et al. (27) and Allen (1).

Infrared analyses of certain compounds were performed on a Perkin-Elmer Model 237 spectrophotometer equipped with beam condensor. Spectrophotometer cells were used with .005 in spacers and methylene chloride as a solvent.

Statistical analyses of paired samples were computed by employing students' distribution (41). Treatment means were compared by using Duncan's multiple range test (11) following analysis of variance.

Table 2. Oven temperature and time relationships for the matrix sequence used in all chromatographic measurements.

Time		Temperature	Program power*
Cumulative	Interval		
(Minutes)		(°C)	
0-10	10	100	50
10-25	15	120	50
25-35	10	140	50
35-50	15	160	50
50-58	8	180	60
58-73	15	200	60
73-77	4	220	60
77---	--	240	60

*Power indicator for programming rate of Aerograph Model 600 D oven.

RESULTS AND DISCUSSION

In 1966, preliminary extractions were made from celery stalks of cultivar Florimart according to the procedures previously discussed. Figure 1 shows chromatograms obtained from the extracts of the liquid nitrogen and dry ice-acetone traps. All peaks are measured at range 1, attenuation 8, unless otherwise designated. Little difference was observed between the component distribution as an effect of the two trapping procedures (liquid nitrogen vs. dry ice-acetone). A larger quantity of extract was obtained from the dry ice-acetone trap than from the liquid nitrogen trap.

High boiling components are designated as those which have retention times greater than 50 minutes: the time at which the oven temperature is set at 180°C. Low boiling fractions are those components which emerge from the column in less than 50 minutes. Figure 2 shows a chromatogram prepared from an extract sample of the aqueous condensate procedure described earlier. Reference to Figure 1 shows that there is a proportionately smaller quantity of high boiling components in the chromatograms from the dry ice-acetone and liquid nitrogen traps than in the chromatogram from the aqueous condensate shown in

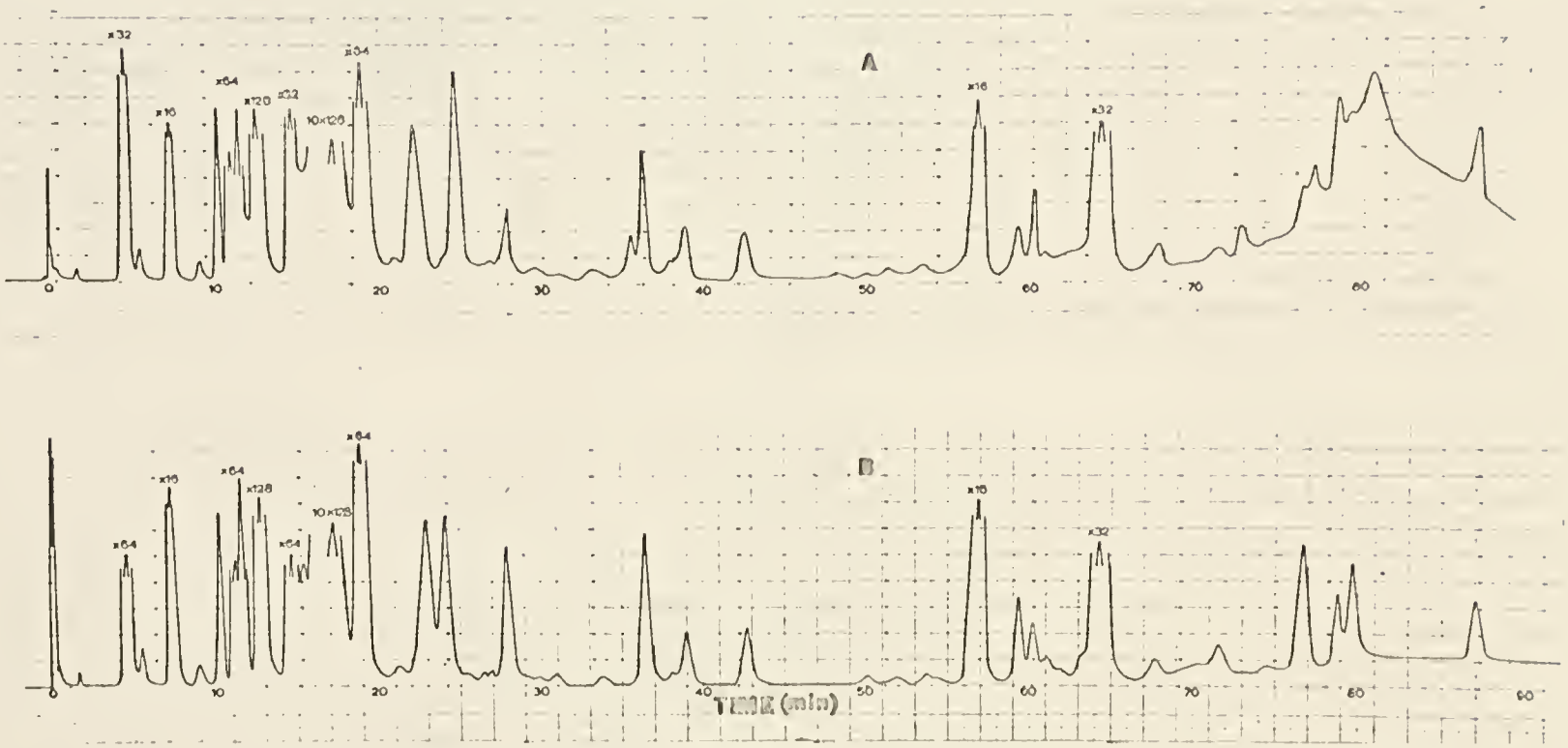

Figure 1. Chromatograms prepared from (A) dry ice-acetone and (B) liquid nitrogen extracts.

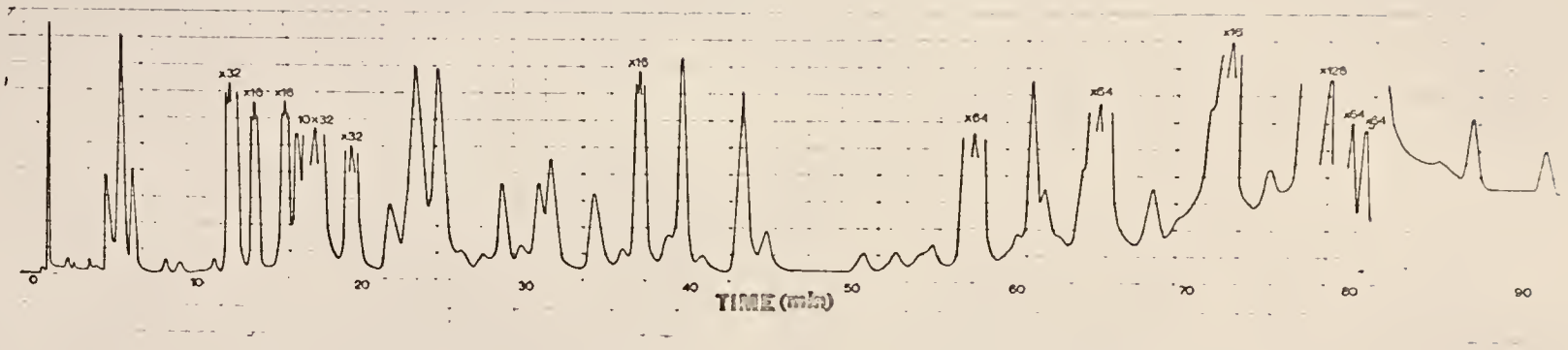

Figure 2. Chromatogram prepared from aqueous extract as described in extraction procedures.

Figure 2. Peaks with retention times of 72 minutes or more made up more than 40 per cent of the total peak area of the chromatograms prepared from these aqueous extracts, while they made up less than 5 per cent of those from the liquid nitrogen and dry ice-acetone traps. While both methods yielded many low boiling compounds, trapping with liquid nitrogen and dry ice-acetone gave a larger proportion.

The data obtained from the chromatograms prepared from the aqueous collections revealed several additional peaks which had retention times greater than 90 minutes, on the Apiezon column, when the instrument was isothermally controlled at 240°C subsequent to the sequence listed in Table 2. The retention times and average peak heights of these peaks are presented in Table 3. These peaks are not considered in treatment comparisons.

In order to establish qualitative meaning of the chromatogram in Figure 2, an odor characterization was established according to the odors noted at the exit port of the column during the programmed sequence. These odors are presented in Table 4 with their estimated strengths. Few celery-like odors were observed in the low boiling range, while a large proportion of the odors in the high boiling range were characteristic of celery. It should be noted that additional odors may have been present but were not detected due to concentration or sensitivity effects.

Since many of the phthalide compounds which impart the typical aroma of celery are sterioisomers (33), it is

Table 3. Retention times on Apiezon L column and average peak heights of peaks with retention time greater than 90 minutes.

Retention time	Average peak height
(Minutes)	(Millimeters)
105	11
108	52
120	31
130	42
142	42
147	17
156	455
170	63
214	42
272	42

Table 4. Odors detected at the exhaust port of an Apiezon L column after injection of 1 ul of celery solvent extract residue.

Time (Minutes)	Odor*	Time (Minutes)	Odor
11	Turpentine (f)	40	Fishy (f)
12	Woody (f)	41	Undescribed (f)
18	Orange (m)	44	Diesel exaust (m)
20	Spinach-sweet (m)	45	Celery (f)
22	Bitter weed (f)	55	Sweet (f)
24	Sour milk (f)	56	Orange peel (m)
25	Plastic glue (m)	58	Cotton (s)
26	Bananas (s)	59	Cotton (f)
27	Green bananas (m)	68	Apple (s)
28	Milk weed (f)	72	Celery (f)
29	Celery (f)	73	Celery (s)
30	Undescribed (f)	74	Apple (f)
31	Undescribed (f)	75	Celery (m)
32	Undescribed (f)	78	Cooked celery (m)
33	Undescribed (f)	79	Celery (m)
36	Undescribed (f)	80	Celery (m)
37	Mint (m)	81	Celery-carrot-like (m)
38	Terpinyl acetate (s)	82	Celery-like (s)
39	Undescribed (f)	85	Cooked celery (f)

*Odor descriptive terms selected by author.

**(f), (m), and (s) designate relative strength of odors as faint, medium, and strong, respectively.

difficult to determine which compound is of most importance. Most of these isomers readily convert to butyl phthalide, resulting in butyl phthalide contamination in any isolation. A sample of n-butyl phthalide which strongly yielded the characteristic aroma of celery was obtained.[1] This compound had a retention time of 77 minutes on Apiezon L and 69 minutes, 30 seconds on Carbowax 20 M. In order to substantiate further the true structure of this compound, an infrared spectrum was prepared. The trace of the infrared spectrum (Figure 3) very closely resembles that presented by Mitsuhashi, et al. (31) for Ligustilide, a sterioisomer of butyl phthalide. However, the spectrum of Figure 3 shows absorption at 3,030 cm^{-1} with 1,600 and 1,535 cm^{-1} indicating the presence of all three double bonds around the benzene ring (3). These data suggest that the compound is n-butyl phthalide rather than Ligustilide or a mixture of neocnidilide and butyl phthalide (33).

On the basis of these data it is assumed that the peak appearing at 77 minutes' retention time from solvent front of Apiezon L is n-butyl phthalide. Collection of the actual peak would have been desirable; however, needed facilities were not available.

When using a fractional collection apparatus, Gold and Wilson (14) found 25 compounds present in the dry ice

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[1]United States Department of Agriculture Fruit and Vegetable Laboratory, Winter Haven, Florida.

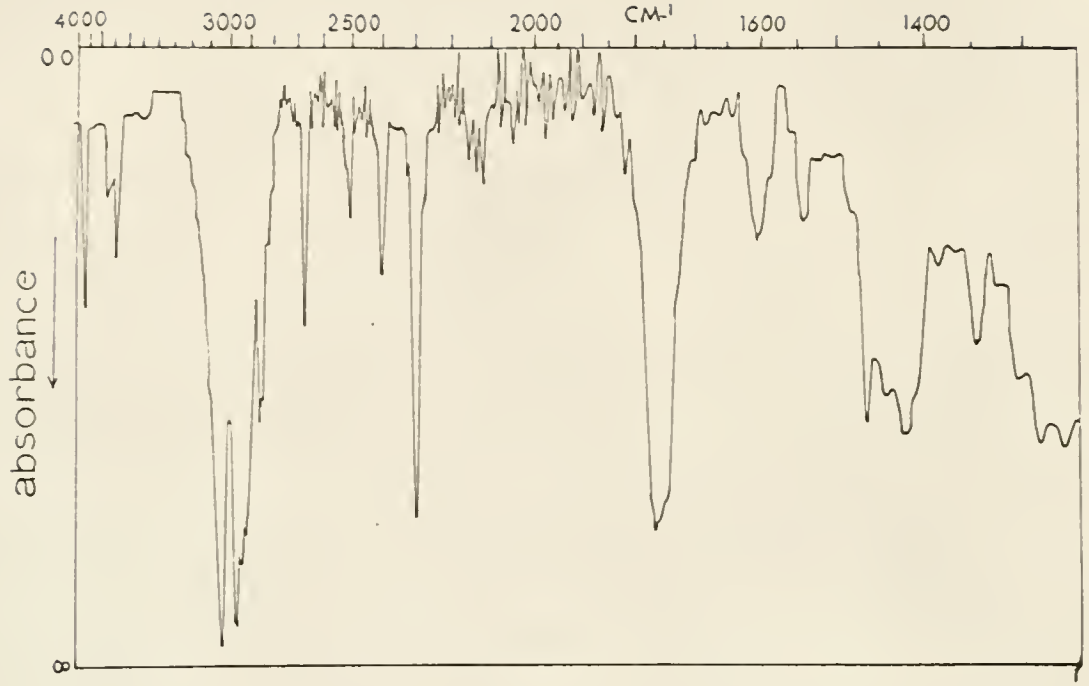


Figure 3. Infrared spectrum of compound believed to be n-butyl phthalide.

trap. Among these compounds they discovered no acids, aldehydes or phenols. They also indicated only 4 compounds were present in the liquid nitrogen condensate, and the principal odor constituent of the dry ice trap was cis-3-hexen-1-yl pyruvate, and that of the liquid nitrogen trap was diacetyl (16). The presence of more compounds in these extracts seems to indicate the fractionating system of Gold and Wilson was more efficient in reducing the vapor temperature than the system used in these experiments.

The importance of phthalides and their derivitives as flavor contributors in celery have been pointed out by several authors (2, 7, 14, 15, 16, 19, 31). It therefore seems essential that a representative celery extract contain a portion of these high boiling phthalide compounds. On the basis of previous investigations (16, 26), the low boiling compounds shown in the previous chromatograms were believed to be primarily $C_{10}H_{16}$ hydrocarbons and related compounds.

The data obtained from the aqueous essence sample are in agreement with those of Gold and Wilson (16). By separating the high boiling and relatively low boiling fractions, they found the phthalides primarily responsible for the celery odor located in the column bottom. They did, however, indicate that the addition of this material to tomato juice did not reproduce the tomato-celery juice blends unless material from the dry ice or liquid nitrogen traps was included. Since the solvent extract prepared from the aqueous essence contains both fractions, it is believed

that the profile shown in Figure 2 most adequately represents the flavor profile of celery.

Organoleptic Evaluation

Triangular taste test comparisons between freshly harvested celery and celery stored 2 weeks at 38°F indicated there was a significant (0.05 level) difference between treatments. Celery used for this first test was harvested on April 24 (stored 2 weeks) and May 9 (fresh). However, when comparison was made between celery harvested May 9 (stored 2 weeks) and May 24 (fresh), no significant difference was observed. In the first test 61 per cent of the judges paired the samples correctly, while in the second test only 39 per cent were able to pair them correctly. From these data it is difficult to determine if differences did exist as a result of storage or if the differences were inherent within each harvest. Hall (21) found that the flavor of celery did change while in storage at 40°F, and that there was an interaction between storage temperature and petiole position on the organoleptic measurement. When comparing Florida-grown with California-grown celery, variations were found between April and May harvests (22). These differences were attributed to a change in sucrose content and not to any large change in the volatile flavor constituents. However, Hall (23) indicated that a taste panel could easily be influenced by the presence of the bitter substance which is associated with the outer petioles.

It is assumed that if differences do exist in the volatile flavor constituents between celery stored 2 weeks at 38°F and freshly harvested celery, storage at higher temperatures and for longer durations should serve to compound these differences. Texture changes in celery stored at higher temperatures make triangular taste tests quite difficult.

Storage

Phase I

Chromatograms used for computation of these data are similar in appearance to the chromatogram shown in Figure 2 and were prepared using the aqueous trapping procedure. There seemed to be little change in the chromatograms from the celery stored 2 or 4 weeks at 38°F when compared to their respective controls.

The importance of the odor characteristics of the high boiling phthalides has already been discussed. However, any true flavor change will be a result of the interactions of all compounds present, whether in threshold or sub-threshold concentrations (18). Changes in the ratio of high/low boiling components will be used as an indicator of change for all chromatograms. Changes in this ratio do not necessarily indicate flavor changes since no research has established a correlation.

The average ratio of high boiling/low boiling components for all chromatographed samples was 1.32. Ratios for high boiling/low boiling fractions for the low temperature

storage treatments are presented in Table 5. The ratio for the freshly harvested sample (1.33) was very near the average for all treatments. While no significant change was observed between freshly harvested samples and those stored 2 weeks at 38°F, there was a slight increase in the mean ratio after 4 weeks' storage.

Table 5. The effect of low temperature (38°F) storage on the ratio of high/low boiling fractions in celery flavor extracts.

Treatment		
Harvest	2 weeks' 38°F	4 weeks' 38°F
	(Fraction ratio-high/low)	
1.33	1.30	1.65

While a large number of peaks were resolved for each chromatogram, only a few contribute substantially to the total peak area of these chromatograms. The 9 peaks which contribute most (more than 1.0 per cent of total area) to the total peak area are listed in Table 6. Also presented in this table is the mean per cent each contributed to the chromatogram total for the respective treatments. The only significant change observed was an increase in the peak at 17 minutes (tentatively identified as d-limonene) when celery was stored 2 weeks at 38°F. This is difficult to explain since the mean for this treatment is higher than the control and the sample taken at 4 weeks' storage. It is noted that there was a large increase in the mean per cent of total area

Table 6. The effect of storage at 38°F for 2 or 4 weeks upon the relative per cent of 9 major chromatogram components.

Retention Time	Treatment		
	At harvest	2 weeks	4 weeks
(Minutes)		(Per cent)	
17	20.3a*	23.4b	20.2a
24	6.9	5.6	6.7
36	3.5	2.5	1.7
56	1.0	1.1	1.0
62	1.8	2.7	2.7
72	11.3	7.7	8.4
78	23.6	31.5	29.9
80	9.9	4.4	7.5
81	3.1	3.4	3.6
		(Total per cent)	
	80.4	82.3	81.7

*Values with differing letters in horizontal rows are significantly different at the 0.05 level according to Duncan's multiple range test.

for the peak at 78 minutes when the celery was stored 2 or 4 weeks as compared with the control. This increase was reflected in an increase in the ratio of high/low boiling components for the 4 weeks' storage treatment; however, increases in the low boiling fraction negated this effect at 2 weeks' storage. No significant difference was observed between the total per cent represented by these 9 peaks.

Chromatograms were prepared from celery extracts at weekly intervals during storage for 10 weeks at 38°F. After celery had been stored 8 weeks at 38°F it was of no market value, and approximately 25 per cent of the petioles had to be removed before the extracts were prepared.

Figure 4 shows a chromatogram prepared from celery stored 10 weeks at 38°F versus freshly harvested celery. Peaks in areas of highest concentration (12 to 22 minutes and 72 to 90 minutes) seemed to have been maintained while peaks in areas of low concentration (24 to 70 minutes) have been reduced or are missing from the chromatogram. Noteworthy is the fact that the low boiling components are present in abundance after storage for long periods of time and regardless of the condition of the celery.

Observation of chromatograms prepared from freshly harvested celery show no peaks of large area between 85 and 95 minutes' retention time. Also, no build-up in concentration was experienced in this range when celery was stored 2 or 4 weeks at 38°F. However, when celery extracts from celery stalks stored 8 weeks or more at 38°F were

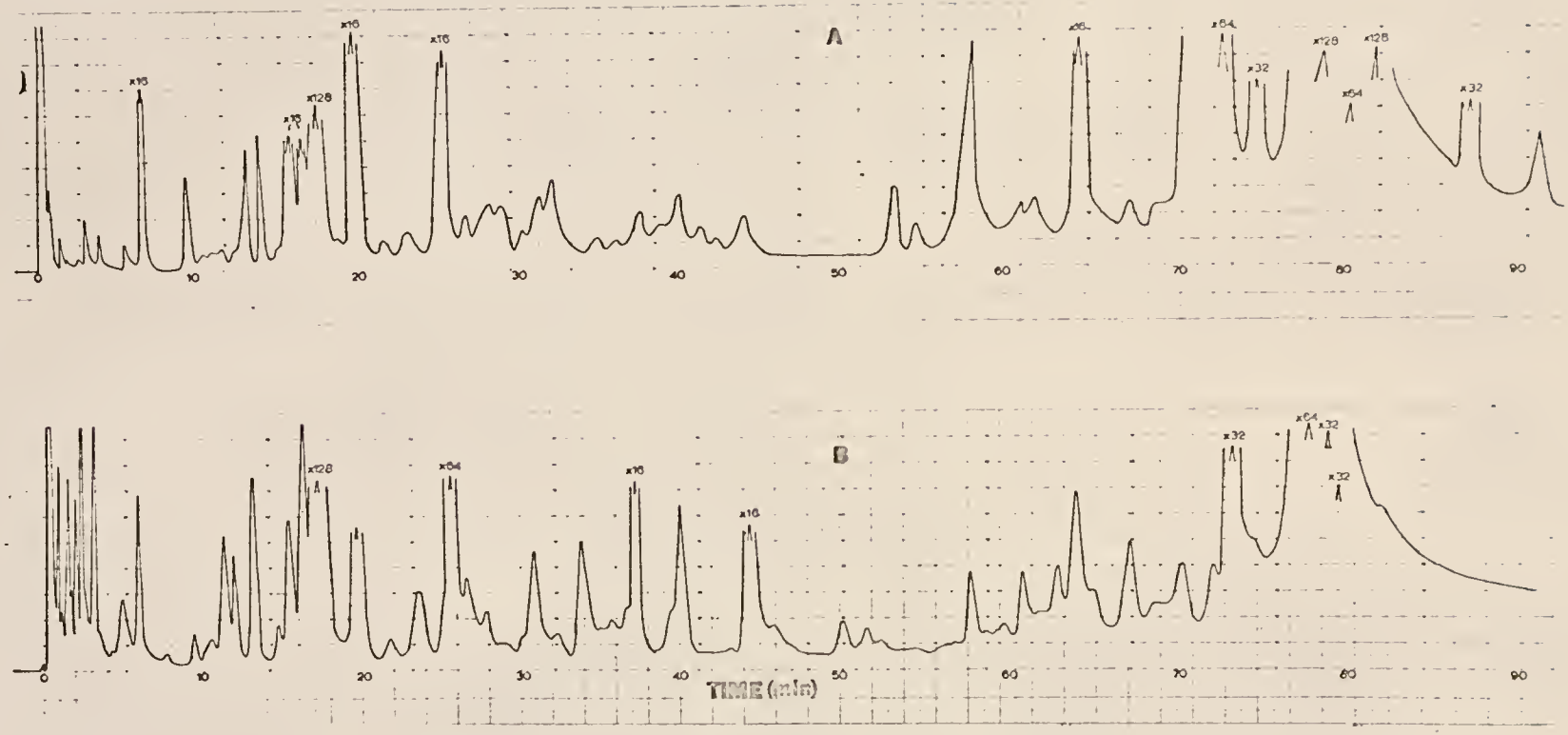


Figure 4. Chromatograms from celery extracts prepared (A) after 10 weeks' storage at 38°F and (B) at harvest.

chromatographed, 2 large (peak height greater than 300 mm) peaks were present at 87 and 91 minutes' retention time.

Celery stored 5 days at 70°F was quite dehydrated and of poor market quality. The freshly harvested control for this study had a high/low component ratio of 0.98, which was lower than the average. This ratio increased to 2.45 when the celery was stored 5 days at 70°F (Figure 5).

This increase in component ratio was accompanied by a corresponding highly significant decrease in total peak area when compared to the freshly harvested control. The mean peak area for the control chromatograms was 48,162.3 mm^2 while the mean peak area from the chromatograms of the 70°F storage treatment was only 23,296.5 mm^2 (Figures 6 and 7). Such a large change in peak area would not normally be expected because the extracts were prepared from a constant volume of juice. Since the moisture content of the celery decreased during storage, it is possible that under these dehydrated conditions a larger proportion of the volatile components remained in the pulp and were not extracted. However, Matthews (30) found a reduction in total volatiles of beans after storage for 5 days at 70°F. A conversion of the volatile flavor components to non-volatile forms through enzymatic action would result in such a change. However, no data have been collected to substantiate this hypothesis.

The changes that occurred in the extracts prepared from celery stored 5 days at 70°F can be seen in Figure 7. There was a decrease in the peak area of all peaks,

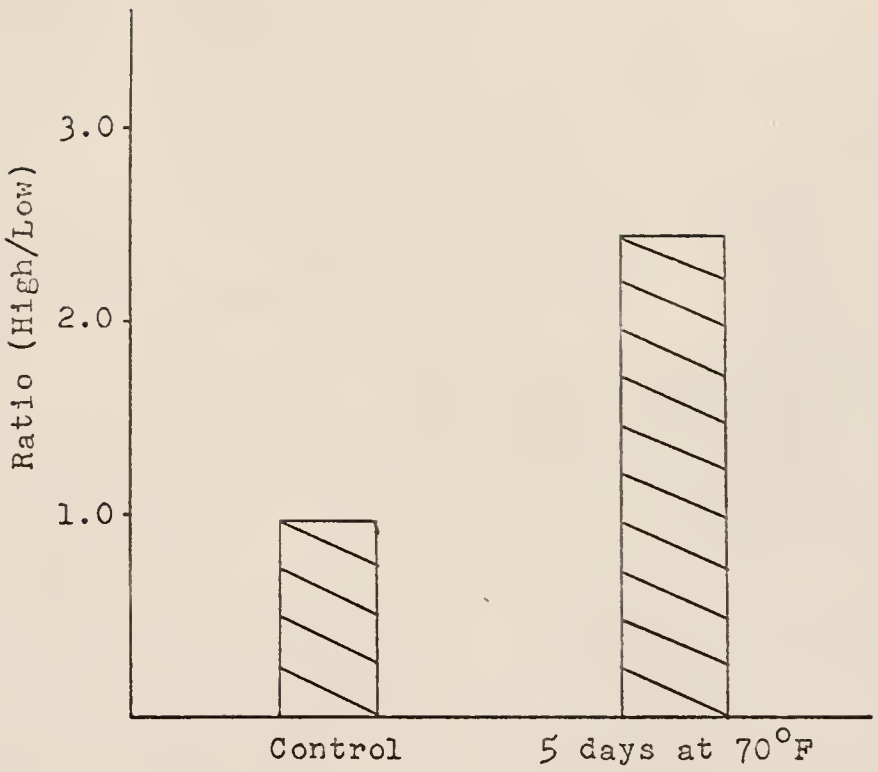


Figure 5. Mean ratio of high/low boiling components as affected by storage for 5 days at 70°F (difference significant at 0.05 level).

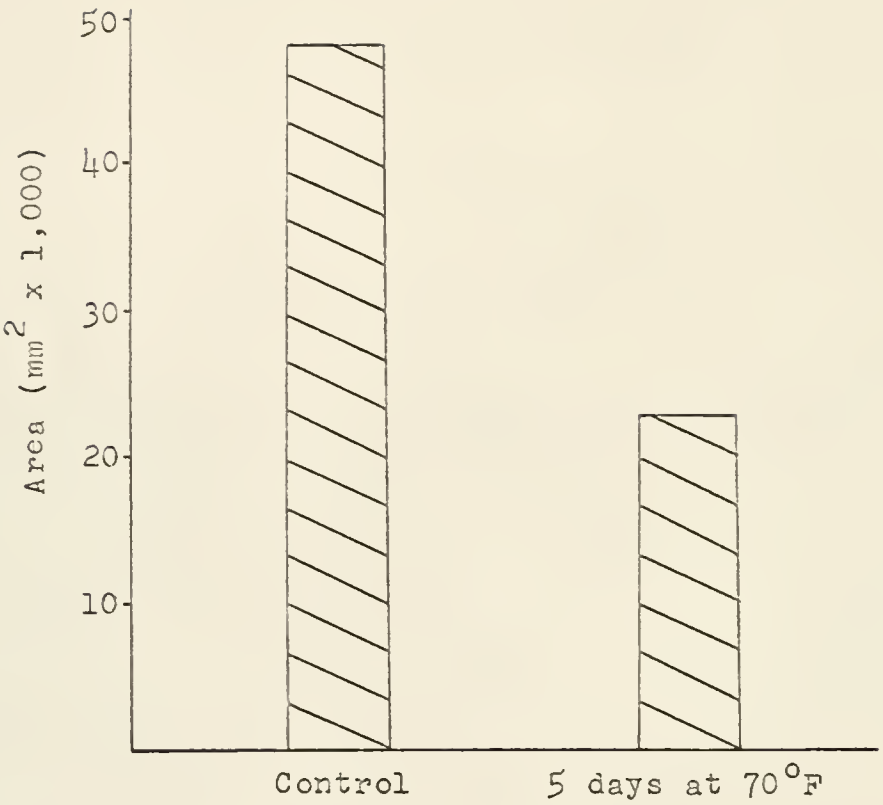


Figure 6. Mean peak area of chromatograms prepared from celery extracts taken at harvest and after 5 days' storage at 70°F (difference significant at 0.01 level).

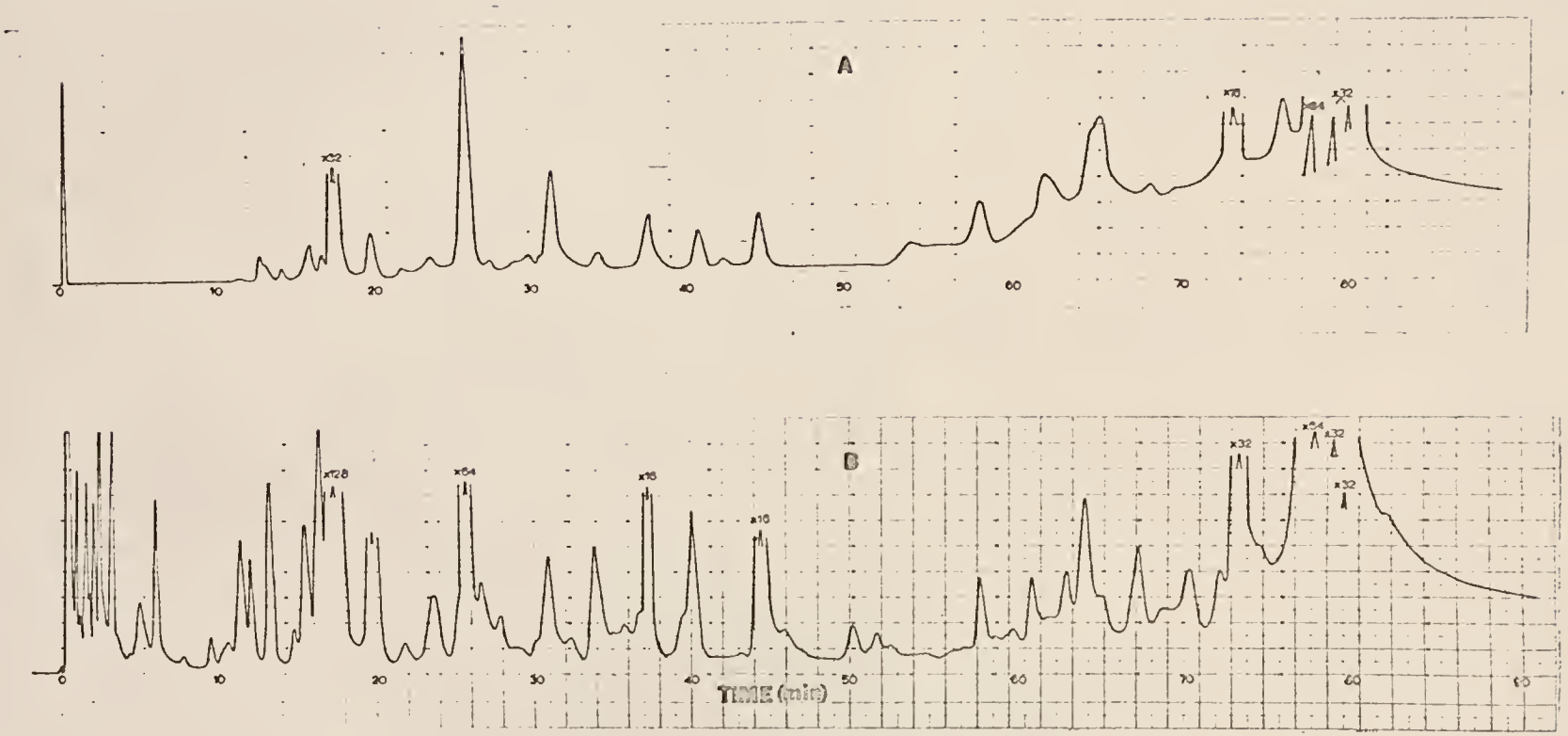


Figure 7. Chromatograms from celery extracts prepared (A) after 5 days' storage at 70°F and (B) at harvest.

particularly those in the low boiling range which appeared before d-limonene (17 minutes). The high boiling components were moderately stable and the peak area decreased only slightly.

Distribution data of the 9 large peaks from the chromatograms prepared at harvest and after 5 days' storage at 70°F are presented in Table 7. There was a significant decrease in the proportion of the total peak area represented by the peak at 17 minutes. Noteworthy is the fact that this change is opposite that observed when celery was stored at 38°F. However, there was a corresponding increase in the per cent represented by the large peak at 78 minutes when the celery was stored 5 days at 70°F. From these data it seems likely that the components of high vapor pressure were metabolized or lost through evaporation as the celery was dehydrated. Table 7 also presents the per cent of the total peak area represented by the sum of the peak areas of the 9 major peaks. The sum of the areas increased when celery was stored 5 days at 70°F. Reference to Table 6 shows that there was a similar increase when celery was stored 2 and 4 weeks at 38°F. This would suggest the possible elimination or decrease of many of the small peaks not considered in these data.

Phase II

Celery used for the market simulation study had a very high peak ratio of high/low boiling components at

Table 7. The effect of storage at 70°F for 5 days upon the relative per cent of 9 major chromatogram components.

Retention Time	Treatment	
	At harvest	70°F for 5 days
(Minutes)	(Per cent)	
17	19.5	9.0*
24	5.7	7.5
36	3.3	1.4
56	1.0	1.6
62	2.1	4.1
72	13.6	8.0
78	22.0	36.9
80	9.3	7.2
81	3.3	9.9
	(Total per cent)	
	79.8	85.6

*Difference significant at the 0.05 level.

harvest as compared to the average for all treatments. The ratio was 2.38 for the freshly harvested celery and decreased significantly (0.05 level) during storage to 1.09 (Figure 8). The peak areas for the treatment means used in computing the high/low ratio are shown in Figure 9. The difference between the areas of each treatment are 21, 39, and 19 per cent for low, high, and total fraction areas, respectively. While there was change in both the low and high component areas, these data indicate a greater change in the high fraction.

A comparison between the mean peak areas of two compounds, d-limonene and n-butyl phthalide (identification based on retention times of known compounds on polar and non-polar chromatographic columns), extracted from the control and market simulated lots of celery, is presented in Figure 10. There was a highly significant increase in the peak area of d-limonene in the chromatograms of the market simulation treatment when compared to those of the control. Considerable variation was observed in the areas of the peak representing butyl phthalide; however, there was a substantial decrease in the mean peak area after storage under market simulated conditions. The changes observed for these two components were in opposite directions.

Any comparison between limonene and butyl phthalide is quite critical from an organoleptic point of view. The importance of the high boiling phthalides has been stressed by the odors noted in Table 4 and by the research of other

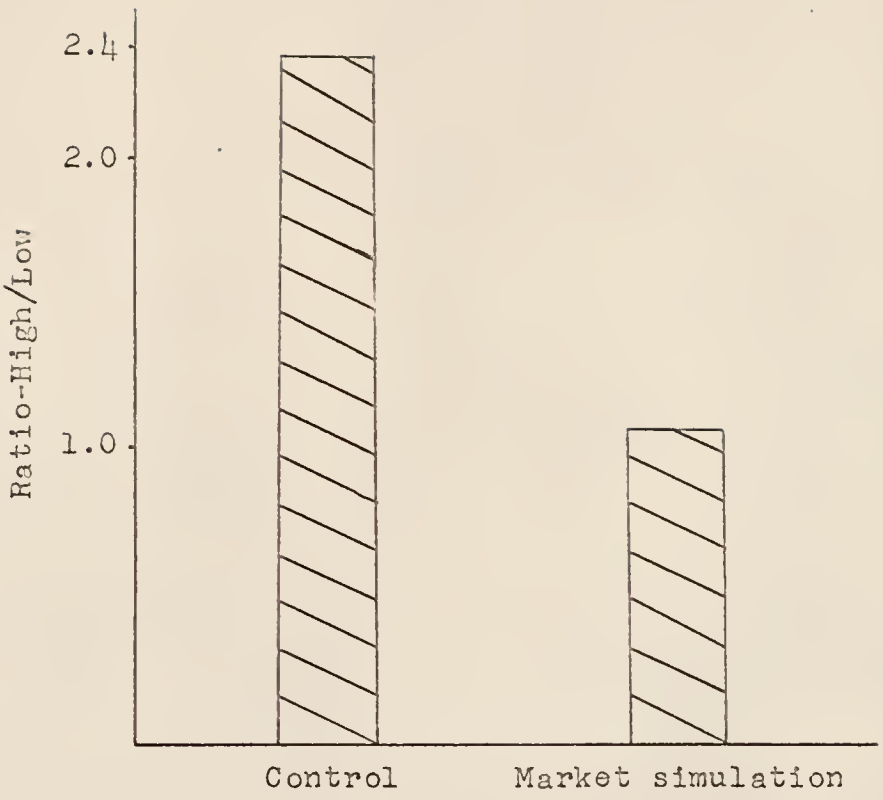


Figure 8. Mean ratio of high/low boiling components of the chromatograms from the control and market simulation treatment (difference significant at 0.05 level).

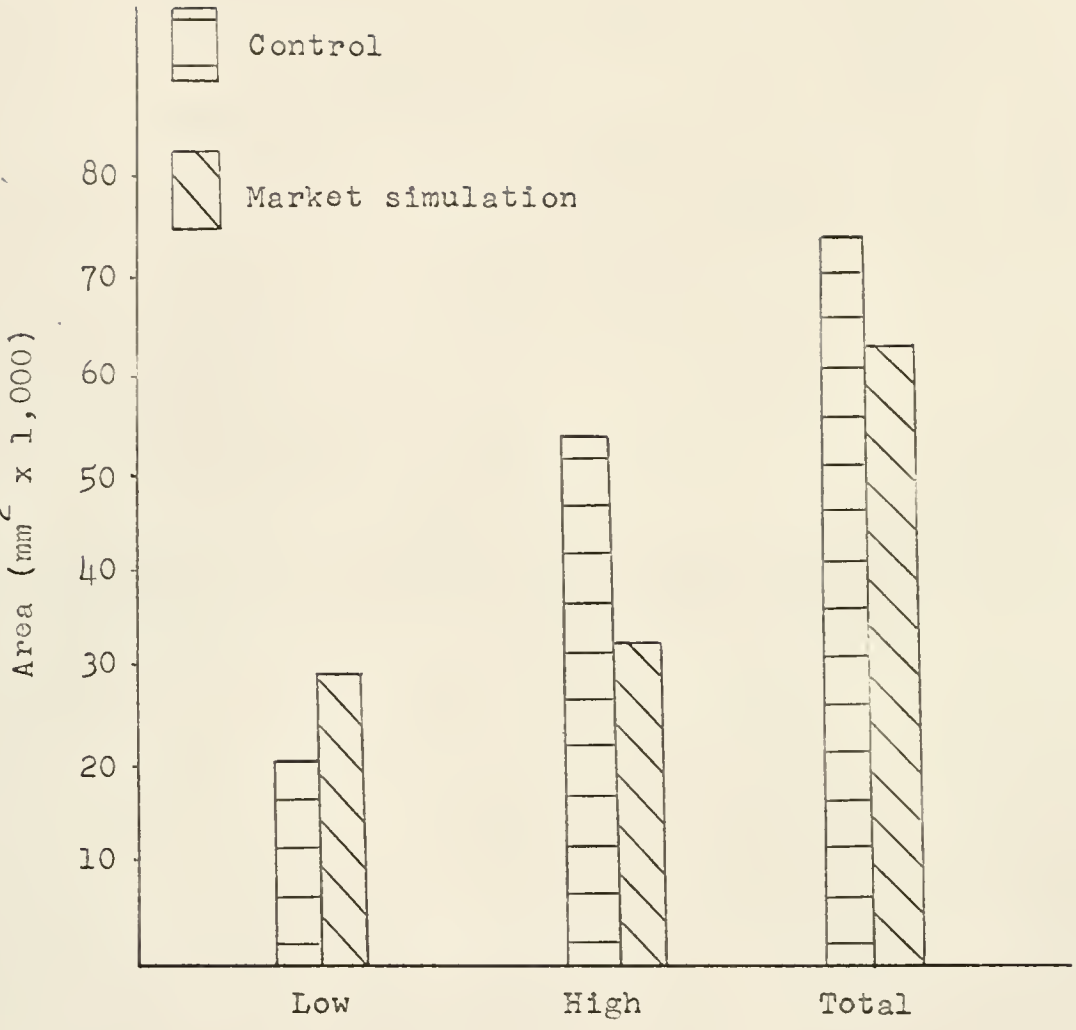


Figure 9. Mean peak area of low and high boiling fractions of the chromatograms of the control and market simulation treatment.

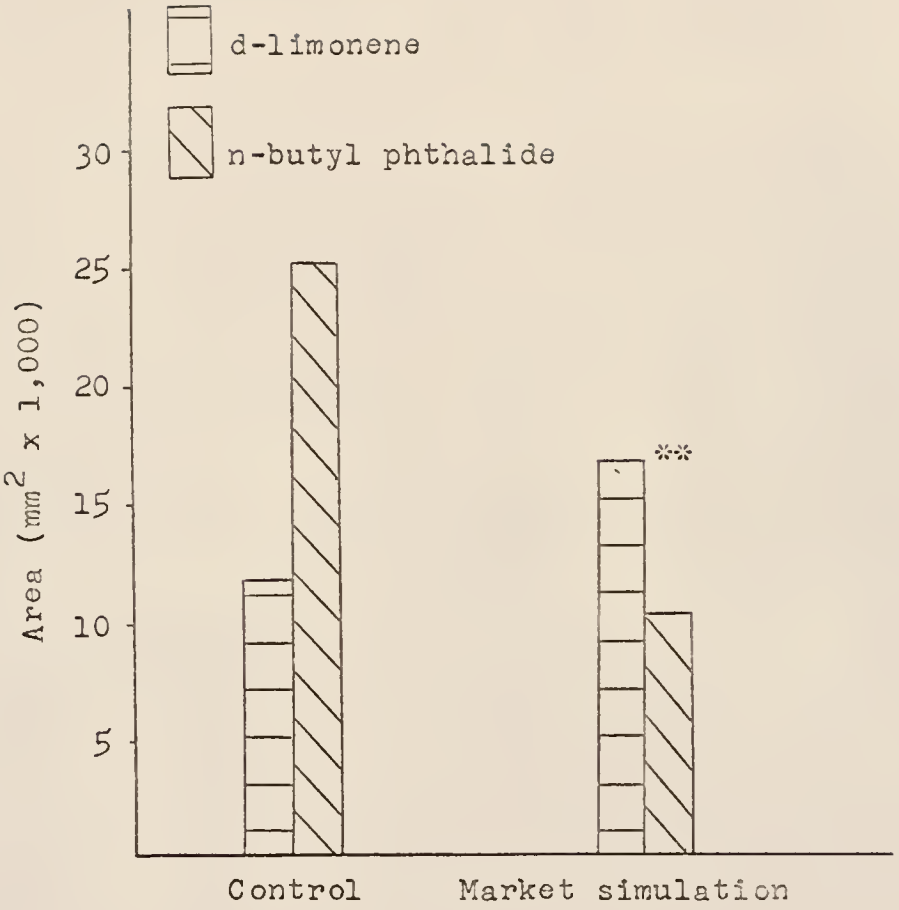


Figure 10. Mean peak area of d-limonene and n-butyl phthalide for the control and market simulation treatment (**change in peak area significant at 0.01 level).

investigators (14, 16). Terpenes, however, seem to be less dominant in determining vegetable flavor than in many of the fruit flavors. It is difficult to determine whether a decrease in the high boiling fraction during storage would result in a decrease in the actual potency of the celery flavor. Any discussion of the decrease in proportion of these elements should take into consideration the additive effects of the various sub-threshold concentrations which are affecting the flavor profile (18). This additive effect of the various components further complicates the discussion of the actual organoleptic effect of a proportionate change in high or low boiling components.

Grouping other peaks of the chromatograms seems to substantiate the change in limonene in the low boiling range. When peaks in the low boiling range (excluding limonene) were considered as a whole, little change was observed between the control and the market simulated study (Figure 11). There was a considerable decrease in the peak area total for those peaks which occurred in the high boiling range (excluding butyl phthalide) as a result of storage under market conditions. It is quite possible that an imbalance of these components at harvest stimulated a change to a more balanced situation. It has not been determined if there could be a conversion of high boiling components to low boiling components during storage, but enzymatic processes are active in the formation of volatile flavor components (25).

In addition to a slight decrease in total peak area

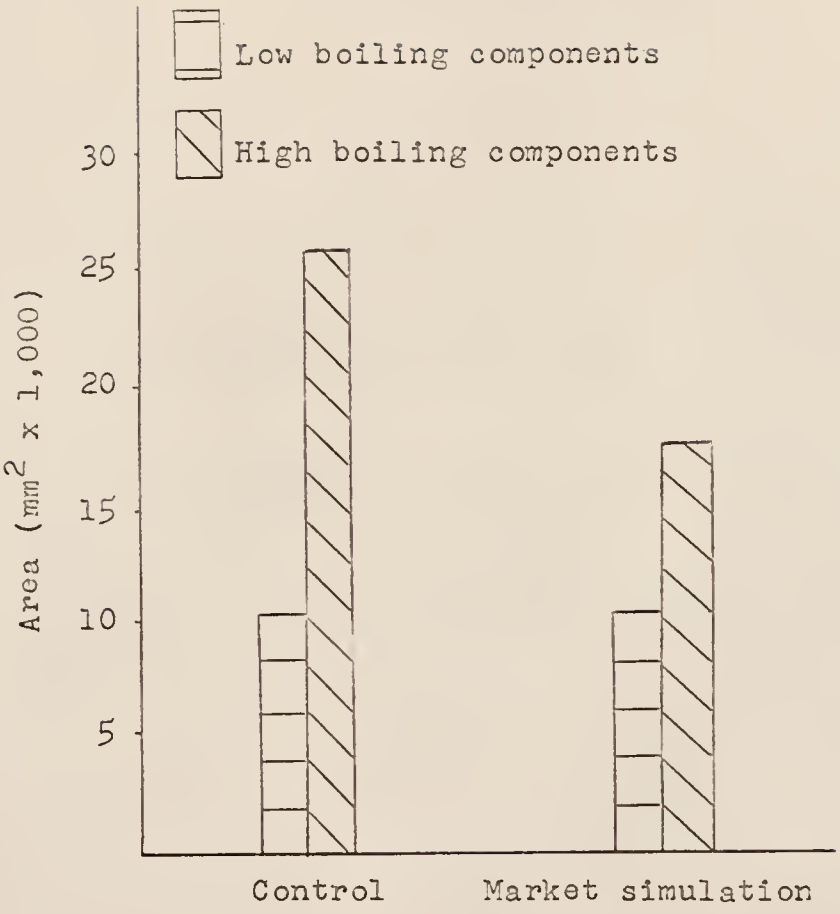


Figure 11. Mean peak area of all peaks in the low and high boiling range excluding limonene and butyl phthalide, respectively.

as a result of storage under market conditions there was a highly significant decrease in the proportionate amount of this total represented by the 9 previously mentioned peaks (Table 8). Also shown is a decrease in the proportionate amount contributed by each of the 9 peaks except peaks at 17 minutes (d-limonene) and at 81 minutes. However, individual peak changes of substantial importance are those at 17, 72, and 78 minutes. Others in combination might also contribute substantially as noted above. These data also indicate a net decrease in those high boiling flavor components most responsible for the aroma of celery.

Chromatograms prepared from the celery extracts of the control and the market simulation treatments are contained in Figure 12. As was pointed out previously, there was an increase in the low boiling fraction, particularly limonene, and a proportionate decrease in the high boiling fraction between 72 and 86 minutes. When celery was stored at 45°F for 2 weeks and 50°F for an additional week, 2 large peaks appeared in the chromatograms at 87 and 91 minutes. The 2 peaks shown in the market simulated treatment chromatogram at 87 and 91 minutes have peak areas of 1,305 mm^2 and 2,004 mm^2 as opposed to 72.3 mm^2 and 176 mm^2 for the chromatogram prepared at harvest. These data are in agreement with those received from chromatograms of celery extracts prepared from celery stored 8 to 10 weeks at 38°F (Figure 4).

Considerable variation occurred in peaks on these

Table 8. The effect of storage under market simulated conditions upon the relative per cent of 9 major chromatogram components.

Retention Time	Control	Market Simulation	Change
(Minutes)	(Per cent)		
	Low boiling		
17	16.6	28.7	+72.9*
24	3.6	3.6	--
36	2.8	2.4	- 7.1
	High boiling		
56	3.4	2.8	-17.6
62	5.8	4.8	-17.2
72	11.9	9.6	-19.3
78	33.5	7.7	-77.0
80	9.8	8.9	- 9.1
81	1.5	2.0	+33.3
	(Total per cent)		
	94.4	80.1**	

*Significant at 0.05 level

**Significant at 0.01 level

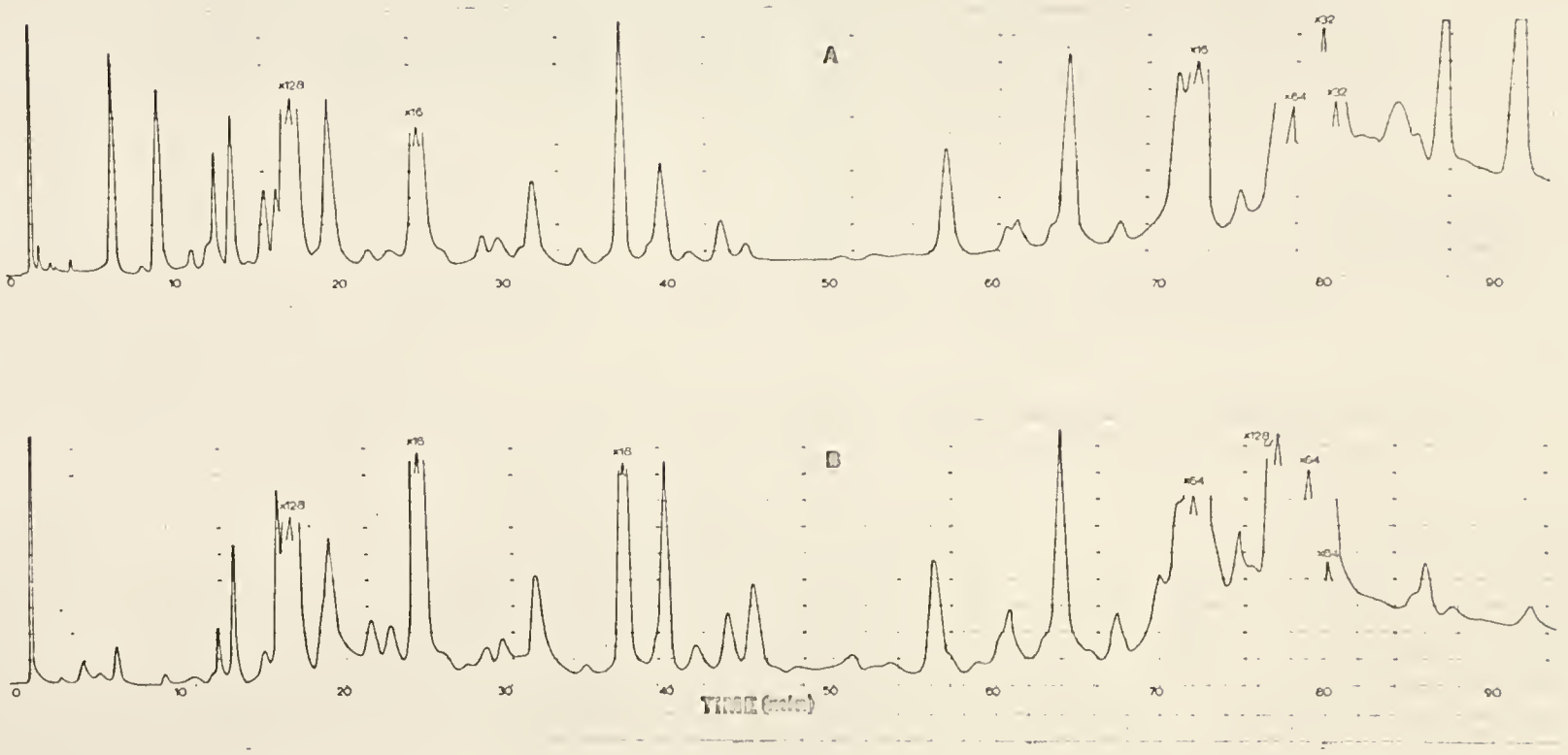


Figure 12. Chromatograms of extracts prepared from (A) celery stored under market simulated conditions and (B) freshly harvested celery.

chromatograms between 37 and 45 minutes' retention time. Peaks in this area were always present in measurable concentrations at range 1, attenuation 8; however, the relative peak areas were not constant within replications.

While the celery used in Phase I and Phase II was of the same chronological age, it is doubtful that the physiological maturity was the same. An indication of this variation could be observed differences between the ratio of high/low boiling components for the freshly harvested samples used for the 70°F storage treatment and for the market simulation. Celery used for the market simulation was harvested in July while that used for the 70°F treatment was harvested in May. If the physiological maturity was not the same for all treatments, generalized comparisons cannot be rendered between treatments (9, 12, 13, 24).

Phase III

Nine separate peaks were resolved from Florida 2-13 celery using the head-space analysis technique. Identification of 4 of these compounds was accomplished by comparisons of their retention times on polar (Carbowax 20 M) and non-polar (Apiezon L) columns and by spiking these chemicals into flasks containing the celery for head-space measurement. Since all comparisons are made with chromatograms from the Apiezon L columns, numbers have been assigned to the resolved compounds on the basis of retention time on this phase (Figure 13). The chromatographic profile obtained

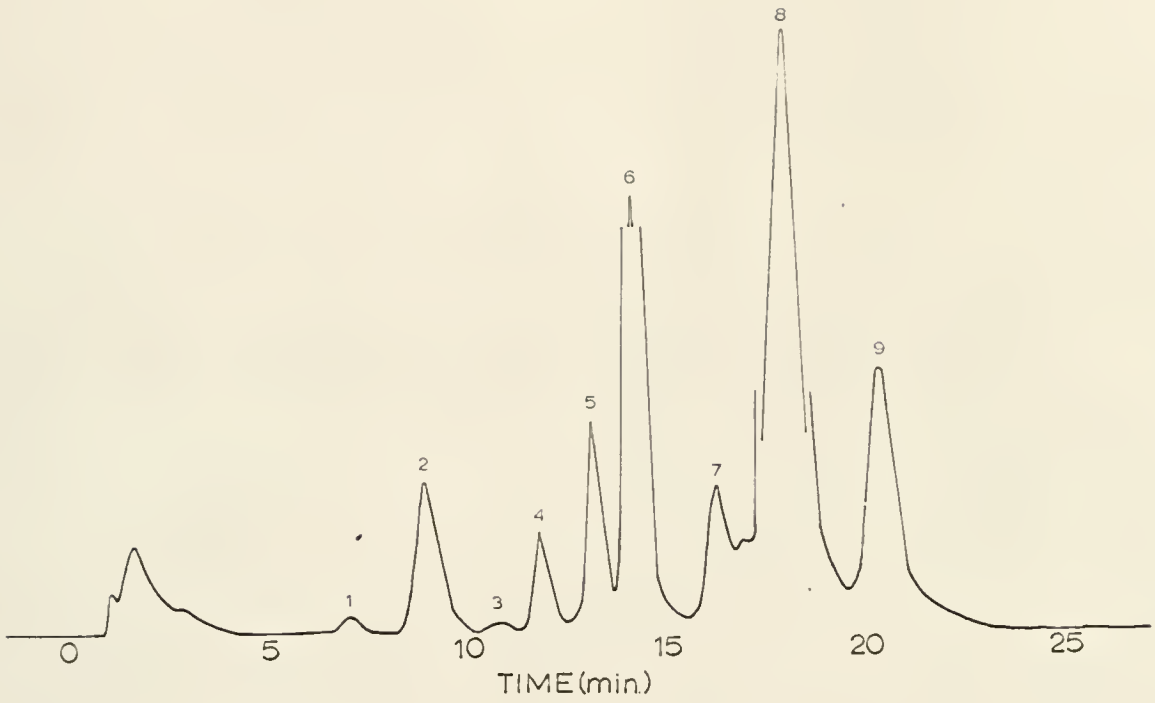


Figure 13. Chromatogram from head-space measurements of celery volatiles with peak numbers according to retention time (Apiezon L).

when these samples were chromatographed on Carbowax 20 M is shown in Figure 14. Comparison data were prepared on the basis of an instrument sensitivity of range 0.1 and attenuation 16, while peaks 6 and 8 were measured at attenuation 32 and 64, respectively, for both columns.

Observed retention times of the 9 volatile constituents are presented in Table 9. These are measured from injection and are somewhat different from those expected when pure compounds are chromatographed. This deviation in time is an effect of the large amount of water vapor present in the holding flask (34). However, the relative retention times are not greatly affected by the presence of water. The retention data presented in Table 9 are in agreement with those of Hunter and Brogden (34). These investigators found α-terpinene to be present in volatile extracts and the location of this compound in their chromatograms substantiates the idea that it is responsible for peak 9. However, no retention data have been collected to support this hypothesis.

The relative per cent of total peak area of the 9 measured peaks is presented in Table 10. Approximately 88 per cent of the total peak area of these chromatograms is accounted for by the four $C_{10}H_{16}$ hydrocarbons identified, while d-limonene and β-pinene resulted in approximately 83 per cent of the total peak area.

The mean peak areas (fresh weight basis) of the 9 peaks are listed in Table 11 according to treatment. Total

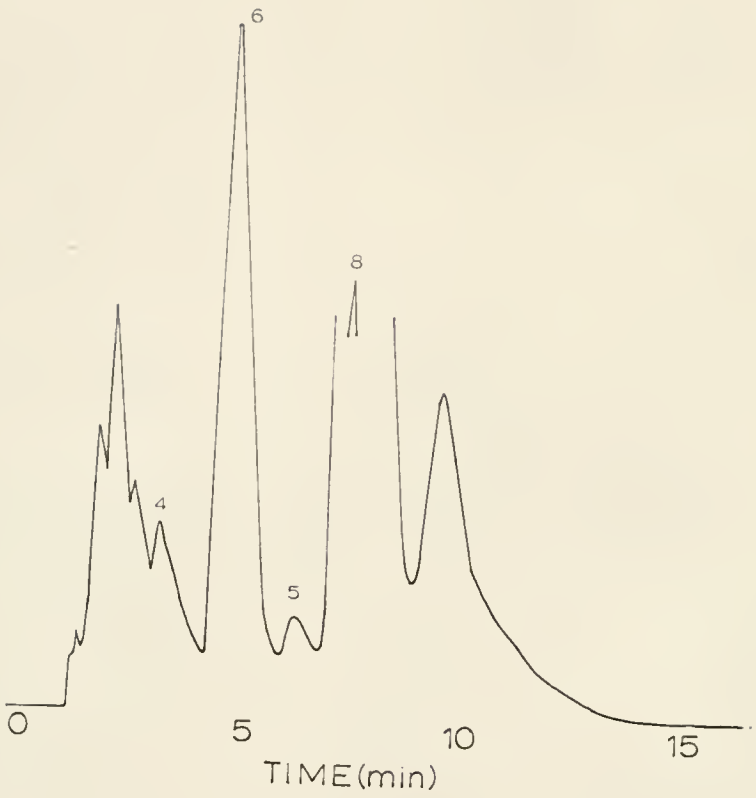


Figure 14. Chromatogram obtained when celery head-space volatiles were chromatographed on Carbowax 20 M.

Table 9. Observed retention times of volatile components of celery head-space samples, using 2 column stationary phases.

Peak Number	Compound	Apiezon L		Carbowax 20 M	
		Unknown	Known	Unknown	Known
		(Minutes)			
1	--	7:00	--	1:10	--
2	--	8:40	--	1:55	--
3	--	10:48	--	1:35	--
4	α-Pinene	11:35	11:35	2:56	2:55
5	β-Myrcene	12:40	12:38	6:00	6:00
6	β-Pinene	13:50	13:50	4:26	4:26
7	--	16:05	--	2:20	--
8	d-Limonene	17:50	17:52	7:15	7:15
9	--	20:20	--	9:23	--

Table 10. Relative per cent of total peak area for the 9 peaks measured in head-space analyses.

Peak number	Per cent of total area
1	0.30
2	2.30
3	0.18
4	1.46
5	2.84
6	11.43
7	3.90
8	72.23
9	5.30

Table 11. Mean peak area (fresh weight basis) for each of the 9 peaks analyzed in head-space measurements as related to storage temperature and duration.

Storage Treatment	Peak number 1	2	3	4
		(Area mm^2)		
Control	16.5a*	277.4a	6.3a	122.2a
1 wk @ 38°F	19.5b	310.4ab	12.3a	145.9ab
2 wk @ 38°F	43.3e	407.5ab	20.4bc	263.5cd
+** 1 day @ 70°F	37.6c	296.5c	16.1abc	177.3ab
+ 8 days @ 50°F	52.4g	310.0ab	25.3c	227.1bcd
2 wk @ 45°F	47.6f	441.1b	27.8c	251.7cd
3 wk @ 38°F	44.6e	389.8ab	25.8c	228.0bcd
4 wk @ 38°F	40.2d	258.7a	22.6bc	199.0abcd
+ 1 day @ 70°F	39.3cd	309.1ab	24.3bc	181.4abc
+ 8 days @ 50°F	58.3h	411.0ab	21.8bc	281.3d
4 wk @ 45°F	47.4f	389.1ab	25.7c	269.8d

*Those means in vertical columns not followed by the same letter are significantly different at the 0.05 level.

**+ designates subsequent storage treatment after storage at 38°F.

Table 11. Continued

Storage Treatment	Peak number 5	6	7
		(Area mm^2)	
Control	220.7a*	1,187.7a	304.9ab
1 wk @ 38°F	306.0ab	1,430.2ab	461.2ab
2 wk @ 38°F	497.0c	1,814.5c	875.9b
+** 1 day @ 70°F	412.5bc	1,532.6abc	593.4ab
+ 8 days @ 50°F	464.9bc	1,803.0bc	642.5ab
2 wk @ 45°F	521.1c	2,141.2c	732.1ab
3 wk @ 38°F	500.5c	1,930.0c	660.8ab
4 wk @ 38°F	406.7abc	1,514.2ab	255.8a
+ 1 day @ 70°F	388.9abc	1,649.0abc	369.0ab
+ 8 days @ 50°F	494.5c	2,137.5c	766.3ab
4 wk @ 45°F	500.8c	1,973.8c	708.3ab

*Those means in vertical columns not followed by the same letter are significantly different at the 0.05 level.

**+ designates subsequent storage treatment after storage at 38°F.

Table 11. Continued

Storage Treatment	Peak number 8	9	Total(1-9)
		(Area mm^2)	
Control	5,454.9a*	552.9a	8,144.1a
1 wk @ 38°F	7,002.3a	571.6abc	10,260.8ab
2 wk @ 38°F	11,650.8a	897.1abcd	16,456.4ab
+** 1 day @ 70°F	11,362.2a	688.0abc	15,117.1ab
+ 8 days @ 50°F	12,149.8a	804.1abcd	16,481.5ab
2 wk @ 45°F	14,232.3a	1,069.2d	19,475.4b
3 wk @ 38°F	11,440.3a	826.6abcd	16,030.1ab
4 wk @ 38°F	11,986.7a	760.7abcd	15,389.5ab
+ 1 day @ 70°F	10,501.9a	666.1abc	14,150.2ab
+ 8 days @ 50°F	12,118.4a	1,459.2e	17,348.0ab
4 wk @ 45°F	14,064.7a	899.1cd	19,381.4b

*Those means in vertical columns not followed by the same letter are significantly different at the 0.05 level.

**+ designates subsequent storage treatment after storage at 38°F.

area for these peaks as a result of treatment is also presented in this table. While all values were not significantly different, the mean peak area for the freshly harvested control was lower than any other treatment for all peaks except peaks 2 and 7. The lowest peak area for these peaks occurred when celery was stored 4 weeks at 38°F. Statistical differences were observed between treatments for all peaks except peak number 8 (limonene).

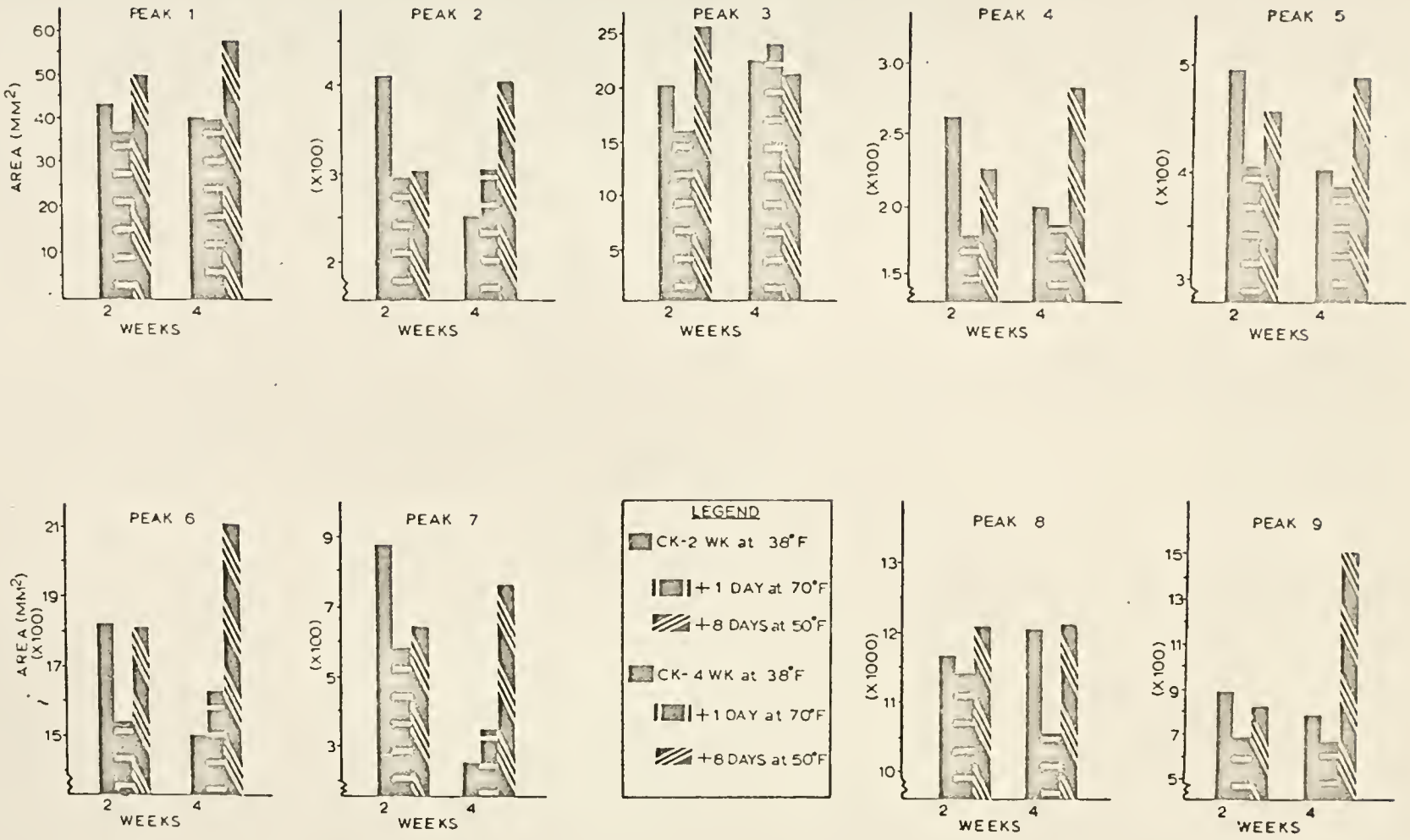
Without exception, the mean peak area of all peaks progressively increased as a result of storage at 38°F for 1 and 2 weeks as compared with the control (Figure 15). This increase in peak area continued for some compounds until the third week of storage; however, all peaks except peak 8 showed a decrease in peak area after 3 weeks' storage at 38°F.

Figure 16 shows a comparison of the volatile changes (FWB) resulting from storage of celery for 1 day at 70°F and 8 days at 50°F after storage for 2 and 4 weeks at 38°F. Considering only the treatments subsequent to 2 weeks' storage at 38°F, without exception, all compounds from the 70°F treatment had a lower peak area than those from the 50°F treatment. There was only one exception to this trend (peak 3) when 70°F and 50°F treatments were prepared subsequent to 4 weeks' storage at 38°F. Also, storage of celery for 1 day at 70°F after 2 weeks at 38°F resulted in a decrease in peak area of all peaks as compared to the freshly harvested sample. The results of the same treatment after 4 weeks at

Figure 15. Trends in the change of peak area (fresh weight basis) as a result of duration in storage at 38°F.


AREA (MM2)
(X100)
(X1000)
14
12
10
8
6
19
17
15
13
11
9
7
5
3
1
50
40
30
20
10
PEAK NO. 8
6
9
5
2
7
4
1
3
0
1
2
3
4
WEEKS

Figure 16. Peak area (fresh weight basis) of celery samples after 2 and 4 weeks' storage at 38°F and subsequent storage after each for 1 day at 70°F and 8 days at 50°F (including all peaks).


PEAK 1
AREA (MM²)
60
50
40
30
20
10
2
4
WEEKS
PEAK 2
(X100)
4
3
2
2
4
WEEKS
PEAK 3
25
20
15
10
5
2
4
WEEKS
PEAK 4
3.0
2.5
2.0
1.5
(X100)
2
4
WEEKS
PEAK 5
5
4
3
(X100)
2
4
WEEKS
PEAK 6
AREA (MM²)
(X100)
21
19
17
15
2
4
WEEKS
PEAK 7
(X100)
9
7
5
3
2
4
WEEKS
LEGEND
CK-2 WK at 38°F
+1 DAY at 70°F
+8 DAYS at 50°F
CK-4 WK at 38°F
+1 DAY at 70°F
+8 DAYS at 50°F
PEAK 8
(X1000)
13
12
11
10
2
4
WEEKS
PEAK 9
(X100)
15
13
11
9
7
5
2
4
WEEKS

38°F are more variable, peaks 4, 5, 8, and 9 showing the decrease after 1 day at 70°F. In some treatments, storage for 8 days at 50°F resulted in an increase in peak area when compared to the corresponding 38°F treatment. These effects were, however, more prevalent after 4 weeks at 38°F (all peaks except peak 2 resulted in the increase).

Peak areas for the control and storage treatments of 2 and 4 weeks at 38°F are presented in Figure 17. There was no significant difference between the two storage treatments at 45°F. A comparison of the 45°F treatments at 2 and 4 weeks reveals that the peak areas resulting from the 45°F treatment tended to be higher than those at 38°F. For both 2 and 4 weeks' storage, the only exceptions were peaks 4, 6, and 7 at 2 weeks, no exceptions being noted at 4 weeks' storage. Noteworthy is the fact that total peak area of all 9 compounds for the 45°F treatments, both 2 and 4 weeks, were the only total values significantly different from the control.

The general trends for these treatments are summarized in Figure 18. The points in this graph are based on the total peak area of all peaks. Storage at 38°F for 1 and 2 weeks resulted in a linear increase in peak area, while further storage at 38°F caused a reduction in peak area. Storage at 70°F for 1 day after storage at 38°F resulted in a decrease in peak area, while storage at 50°F for 8 days after 38°F holding resulted in an increase in peak area. The degree of increase at 50°F was greatly

Figure 17. Peak area (fresh weight basis) of celery samples at harvest, after storage at 38°F for 2 and 4 weeks, and after storage at 45°F for 2 and 4 weeks for each of the 9 peaks measured in head-space analyses.

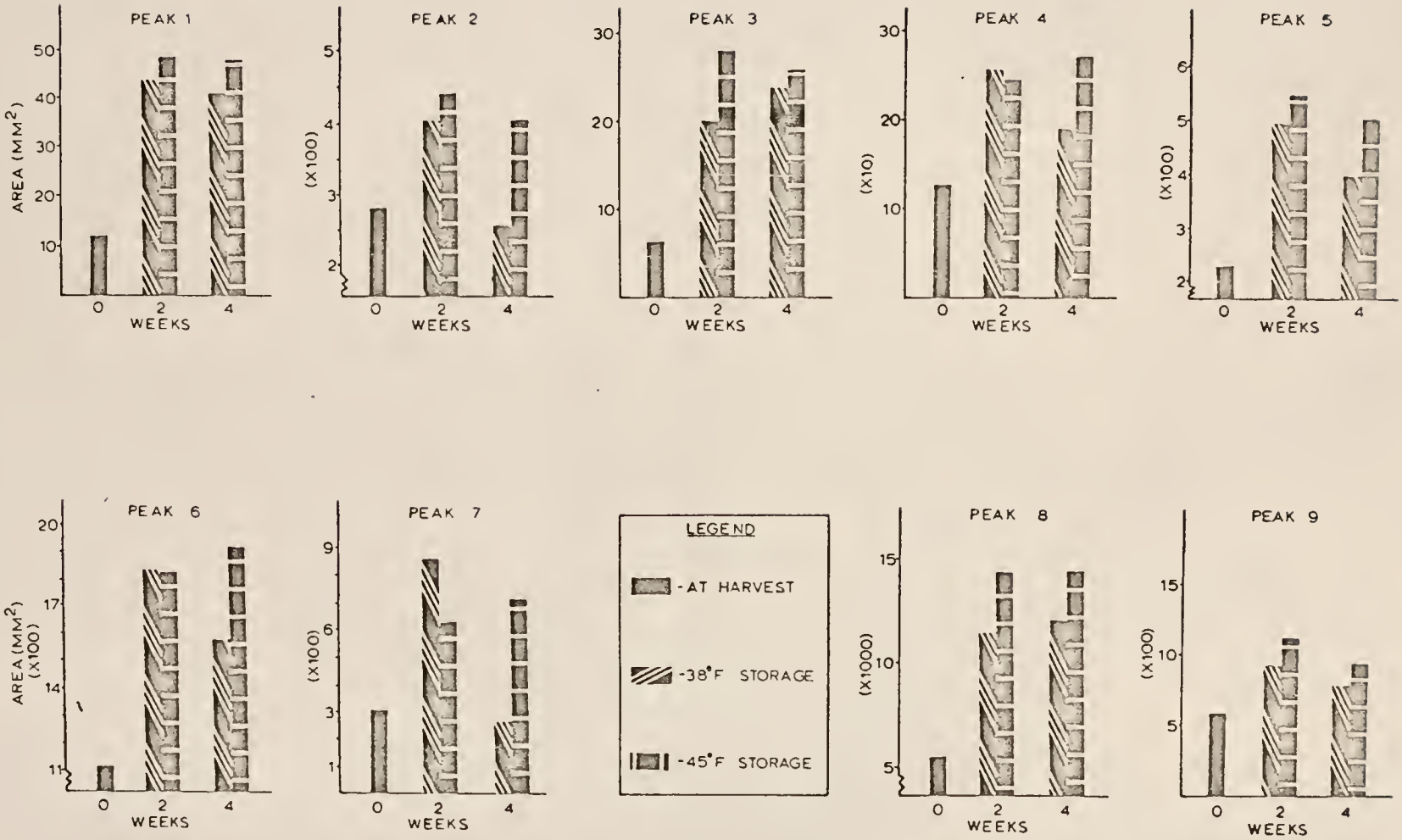

PEAK 1
AREA (MM²)
50
40
30
20
10
0
2
4
WEEKS
PEAK 2
(X100)
5
4
3
2
PEAK 3
30
20
10
PEAK 4
(X10)
PEAK 5
(X100)
6
PEAK 6
AREA (MM²)
(X100)
20
17
14
11
PEAK 7
9
6
3
1
LEGEND
-AT HARVEST
-38°F STORAGE
-45°F STORAGE
PEAK 8
(X1000)
15
10
5
PEAK 9

Figure 18. General trends in total peak area (fresh weight basis) changes according to temperature and duration of storage at 45°F, 38°F, and subsequent storage at 50°F and 70°F after storage at 38°F.

affected by the increase and large value of peak area for peak 8. The greatest total peak areas were observed from celery stored 2 or 4 weeks at 45°F. Interpolation of the change between harvest and 2 weeks' storage at 45°F is not possible for these data.

After one experimental block had been analyzed, there appeared to be differences associated with apparent moisture loss. In attempts to define these effects, dry weight measurements were prepared from celery in the second and third block and these data are presented in Table 12. Few significant differences were observed as a result of storage treatment. However, the per cent dry weight of celery stored 2 and 4 weeks at 45°F was significantly higher than most other storage treatments. Also, the control was significantly different from only 2 treatments: 2 weeks' storage at 45°F and 3 weeks' storage at 38°F.

Since these differences in dry weight were observed, the peak areas were calculated on a dry weight basis (DWB) and are presented in Table 13. When calculated on the basis of dry weight, there seemed to be more variation in the peak areas than when calculated on a fresh weight basis. The peak area (DWB) for the control was not lowest; instead, there was a general decrease in 4 of the compounds to 1 week storage at 38°F (Figure 19). After this storage duration, the peak areas of the various peaks increased as when the data were calculated on a fresh weight basis. All peaks increased in peak area from 1 week to 2 weeks' storage at

Table 12. Per cent dry weight of celery as related to storage temperature and duration.

Storage Treatment	Dry weight
	(Per cent)
Control	4.11bcd*
1 wk @ 38°F	3.99abc
2 wk @ 38°F	3.95abc
+**1 day @ 70°F	4.19bcd
+ 8 days @ 50°F	3.94abc
2 wk @ 45°F	4.82e
3 wk @ 38°F	3.65a
4 wk @ 38°F	3.78abc
+ 1 day @ 70°F	3.67ab
+ 8 days @ 50°F	4.17bcd
4 wk @ 45°F	4.60de

*Those means in vertical columns not followed by the same letter are significantly different at the 0.05 level.

**+ designates subsequent storage treatment after storage at 38°F.

Table 13. Mean peak area (dry weight basis) for each of the 9 peaks analyzed in head-space measurements as related to storage temperature and duration.

Storage Treatment	Peak number 1	2	3	4
		(Area mm^2)		
Control	0.4a*	17.7ab	0.3a	6.0abc
1 wk @ 38°F	0.5a	17.1ab	0.4a	4.9a
2 wk @ 38°F	1.2ab	19.0ab	0.8bc	9.2d
+**1 day @ 70°F	0.9ab	16.0ab	0.7ab	5.6ab
+ 8 days @ 50°F	1.3ab	21.0ab	1.2c	8.7cd
2 wk @ 45°F	1.0ab	21.2ab	1.2c	8.0bcd
3 wk @ 38°F	1.0ab	24.1b	0.8bc	8.6cd
4 wk @ 38°F	0.9ab	14.5a	0.7ab	8.0bcd
+ 1 day @ 70°F	1.3ab	18.9ab	1.0bc	8.4cd
+ 8 days @ 50°F	1.6b	22.4ab	1.0bc	10.6d
4 wk @ 45°F	0.8ab	18.5ab	1.0bc	8.4cd

*Those means in vertical columns not followed by the same letter are significantly different at the 0.05 level.

**+ designates subsequent storage treatment after storage at 38°F.

Table 13. Continued

Storage Treatment	Peak number 5	6	7
		(Area mm^2)	
Control	9.6ab*	68.9ab	9.8ab
1 wk @ 38°F	8.3a	60.6a	5.3a
2 wk @ 38°F	14.9cd	72.2ab	10.8ab
+**1 day @ 70°F	10.9abc	64.2ab	8.2ab
+ 8 days @ 50°F	13.6bcd	74.8ab	9.9ab
2 wk @ 45°F	15.0cd	82.0ab	12.0b
3 wk @ 38°F	15.7d	92.5b	11.3ab
4 wk @ 38°F	11.7abcd	67.1ab	8.2ab
+ 1 day @ 70°F	13.8bcd	72.7ab	9.7ab
+ 8 days @ 50°F	15.1cd	91.6b	13.3b
4 wk @ 45°F	13.9cd	75.2ab	11.6ab

*Those means in vertical columns not followed by the same letter are significantly different at the 0.05 level.

**+ designates subsequent storage treatment after storage at 38°F.

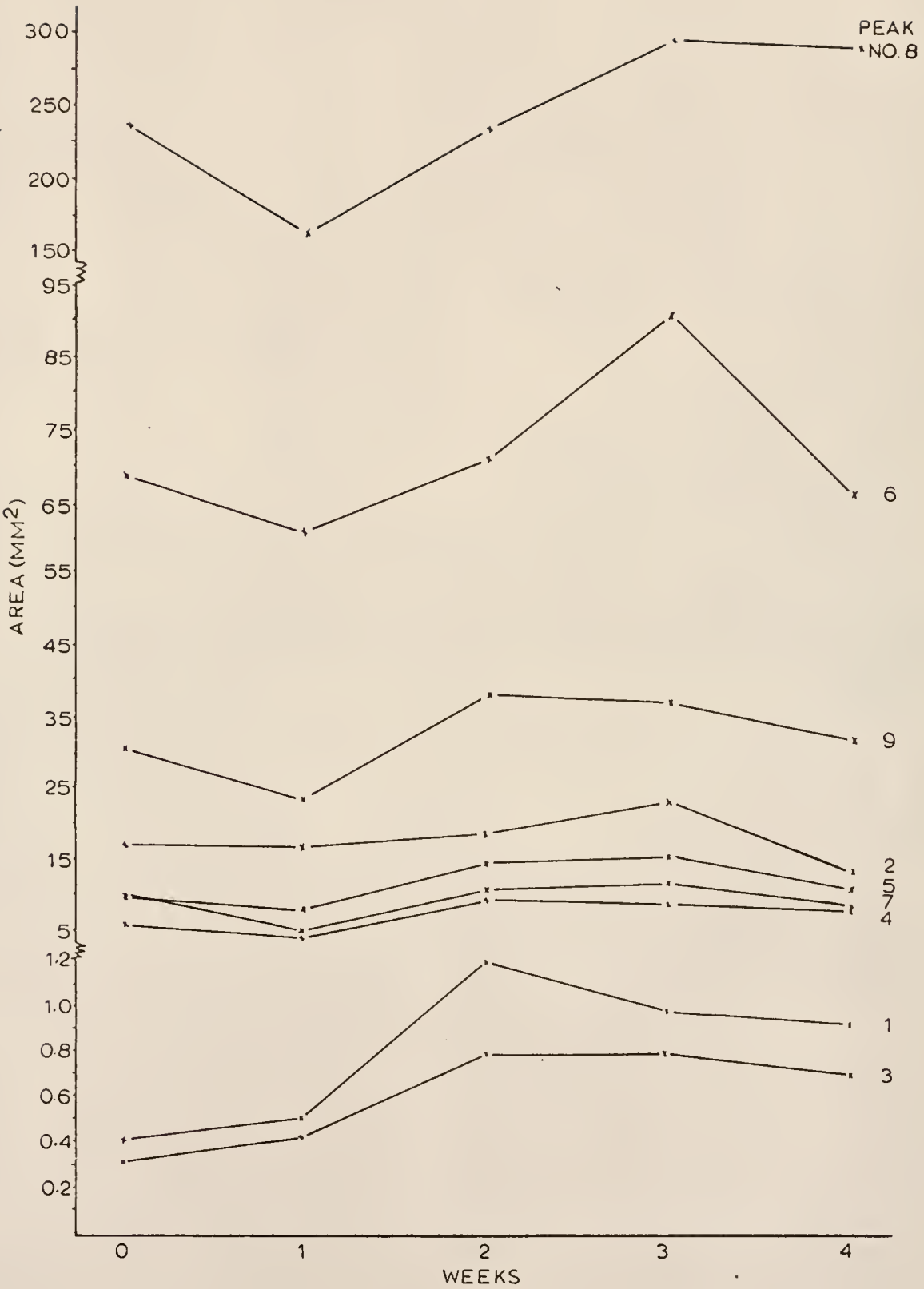
Table 13. Continued

Storage Treatment	Peak number 8	9	Total(1-9)
	(Area mm^2)		
Control	233.2ab*	30.4abc	376.4ab
1 wk @ 38°F	164.3a	23.4a	284.8a
2 wk @ 38°F	232.4ab	37.5cd	397.9ab
+**1 day @ 70°F	251.7ab	27.8ab	386.0ab
+ 8 days @ 50°F	279.8ab	34.1bc	444.5b
2 wk @ 45°F	318.5b	45.2d	505.2b
3 wk @ 38°F	301.6b	36.0bcd	491.6b
4 wk @ 38°F	293.7b	31.1abc	431.6ab
+1 day @ 70°F	286.2b	37.0bcd	450.6b
+ 8 days @ 50°F	315.4b	35.6bcd	506.7b
4 wk @ 45°F	321.4b	37.6cd	488.6b

*Those means in vertical columns not followed by the same letter are significantly different at the 0.05 level.

**+ designates subsequent storage treatment after storage at 38°F.

Figure 19. Trends in the change of peak area (dry weight basis) as a result of duration in storage at 38°F.


PEAK NO. 8
6
9
2
5
7
4
1
3
AREA (MM2)
300
250
200
150
95
85
75
65
55
45
35
25
15
5
1.2
1.0
0.8
0.6
0.4
0.2
0
1
2
3
4
WEEKS

38°F. Also, all peaks showed a decrease in peak area from 3 to 4 weeks when storage was at 38°F.

Figure 20 shows a comparison of the volatile changes (DWB) resulting from storage of celery for 1 day at 70°F and 8 days at 50°F after storage for 2 or 4 weeks at 38°F. Considering only the treatments subsequent to 2 weeks' storage at 38°F, without exception, all peaks from the 70°F treatment had a lower peak area than those from the 50°F treatment. This trend was not true for peaks 3 and 9 when celery was sampled after 4 weeks at 38°F. Also, storage of celery for 1 day at 70°F after 2 weeks at 38°F resulted in a decrease in peak area for all compounds. The results of the same treatment subsequent to 4 weeks at 38°F do not agree with those after 2 weeks at 38°F when compared with the 38°F check at 4 weeks' storage. All peaks except peaks 3, 8, and 9 showed a progressive increase in peak area for 70°F and 50°F storage, respectively. This trend was noted in peaks 2, 6, and 7 for the data calculated on a fresh weight basis.

Peak areas (DWB) for the control and storage treatments of 2 and 4 weeks at 38°F and 45°F are presented in Figure 21. All peaks except 1, 4, and 5 showed an increase in peak area when stored at 45°F (2 and 4 weeks) as compared to 38°F storage. The differences between these treatments and the control are not as great as when the data were calculated on a fresh weight basis.

When considering the peaks on a dry weight basis, the celery stored 2 and 4 weeks at 45°F did not result in the

Figure 20. Peak area (dry weight basis) of celery samples after 2 and 4 weeks' storage at 38°F and subsequent storage after each for 1 day at 70°F and 8 days at 50°F (including all peaks).

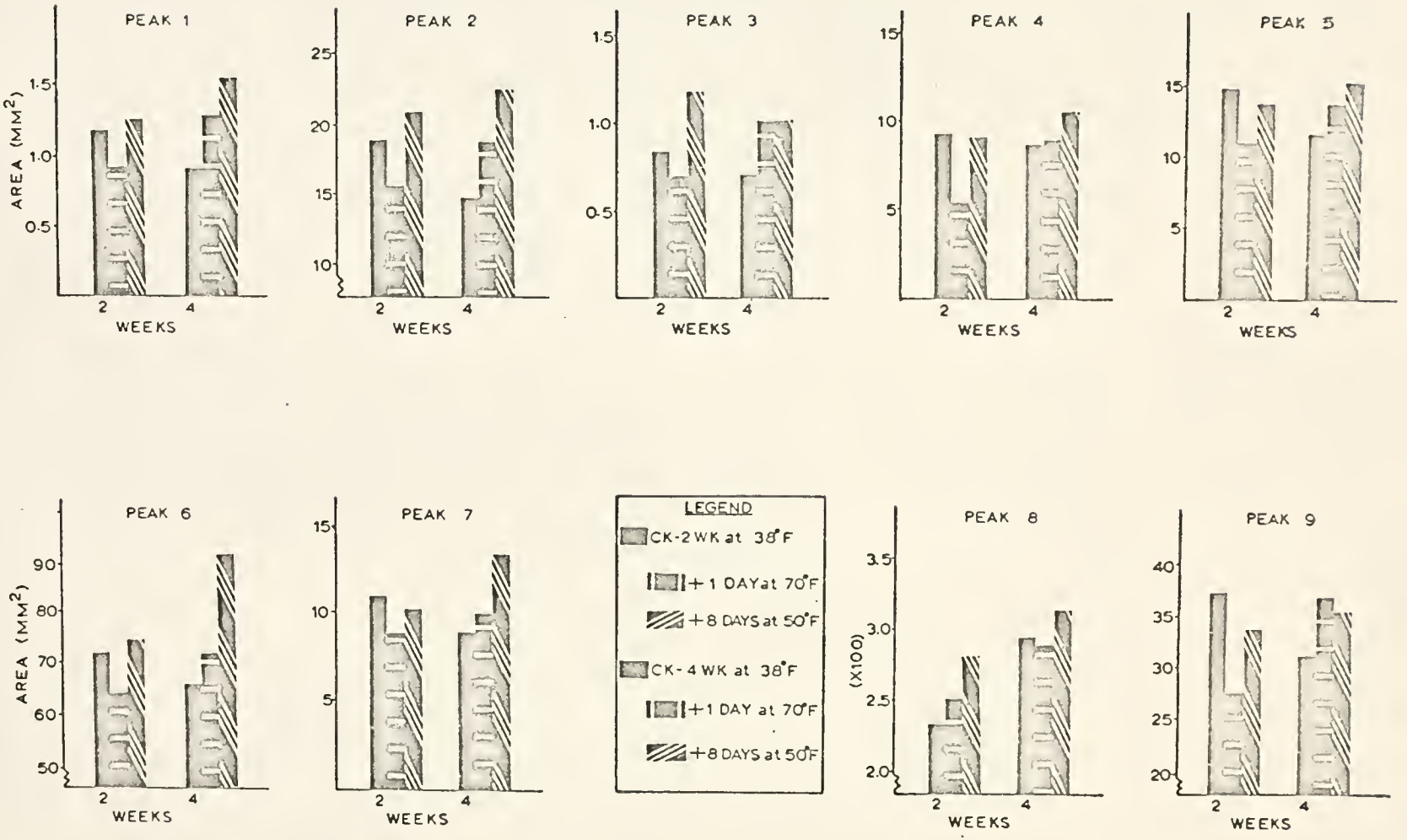
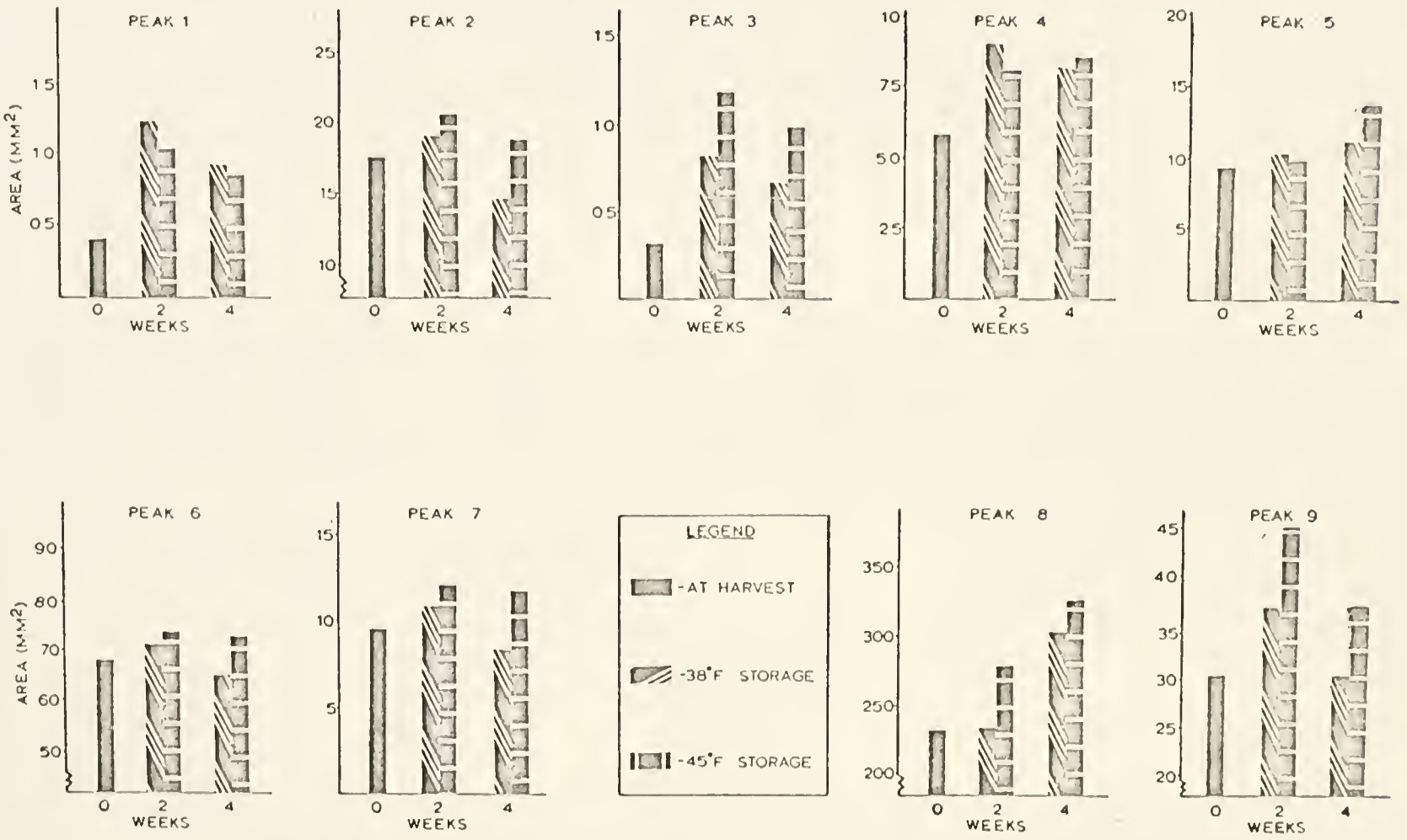

PEAK 1
PEAK 2
PEAK 3
PEAK 4
PEAK 5
PEAK 6
PEAK 7
PEAK 8
PEAK 9
AREA (MM²)
(X100)
WEEKS
LEGEND
CK-2 WK at 38°F
+1 DAY at 70°F
+8 DAYS at 50°F
CK-4 WK at 38°F
+1 DAY at 70°F
+8 DAYS at 50°F

Figure 21. Peak area (dry weight basis) of celery samples at harvest, after storage at 38°F for 2 and 4 weeks, and after storage at 45°F for 2 and 4 weeks for each of the 9 peaks measured in head-space analyses.


PEAK 1
PEAK 2
PEAK 3
PEAK 4
PEAK 5
PEAK 6
PEAK 7
PEAK 8
PEAK 9
AREA (MM2)
WEEKS
0
2
4
LEGEND
-AT HARVEST
-38°F STORAGE
-45°F STORAGE

highest area for all peaks; however, the total peak area for these treatments in each case was higher than the corresponding treatment at 38°F.

A summary of the net effects of these variations is presented in Figure 22. This graph varies from that presented in Figure 18 in two ways. First, there was not a progressive increase in the total peak area from harvest through 2 weeks' storage at 38°F when calculated on a dry weight basis. There was, however, an increase in the total peak area from 1 through 3 weeks' storage at 38°F with a decrease after storage for 4 weeks. Second, when calculated on a dry weight basis, the trend of lowering peak area with storage of celery at 70°F was not consistent. The same decrease was noted in these data as in those calculated on a fresh weight basis when celery was stored 1 day at 70°F after 2 weeks' storage at 38°F; however, when subsequent storage at 70°F was performed after storage for 4 weeks at 38°F, the peak area (DWB) increased slightly. This reflects the inconsistency of peak 8 (Figure 20) which was the only peak showing a decrease after 2 weeks' storage at 38°F and 1 day at 70°F. Those treatment effects experienced at 45°F and at 50°F for fresh weight determinations were also true when those data were calculated on a dry weight basis. Peak areas for 45°F treatments remained higher than any 38°F treatment and treatment at 50°F for 8 days after 38°F storage resulted in increases in peak area.

Considering the peak area on a fresh weight basis,

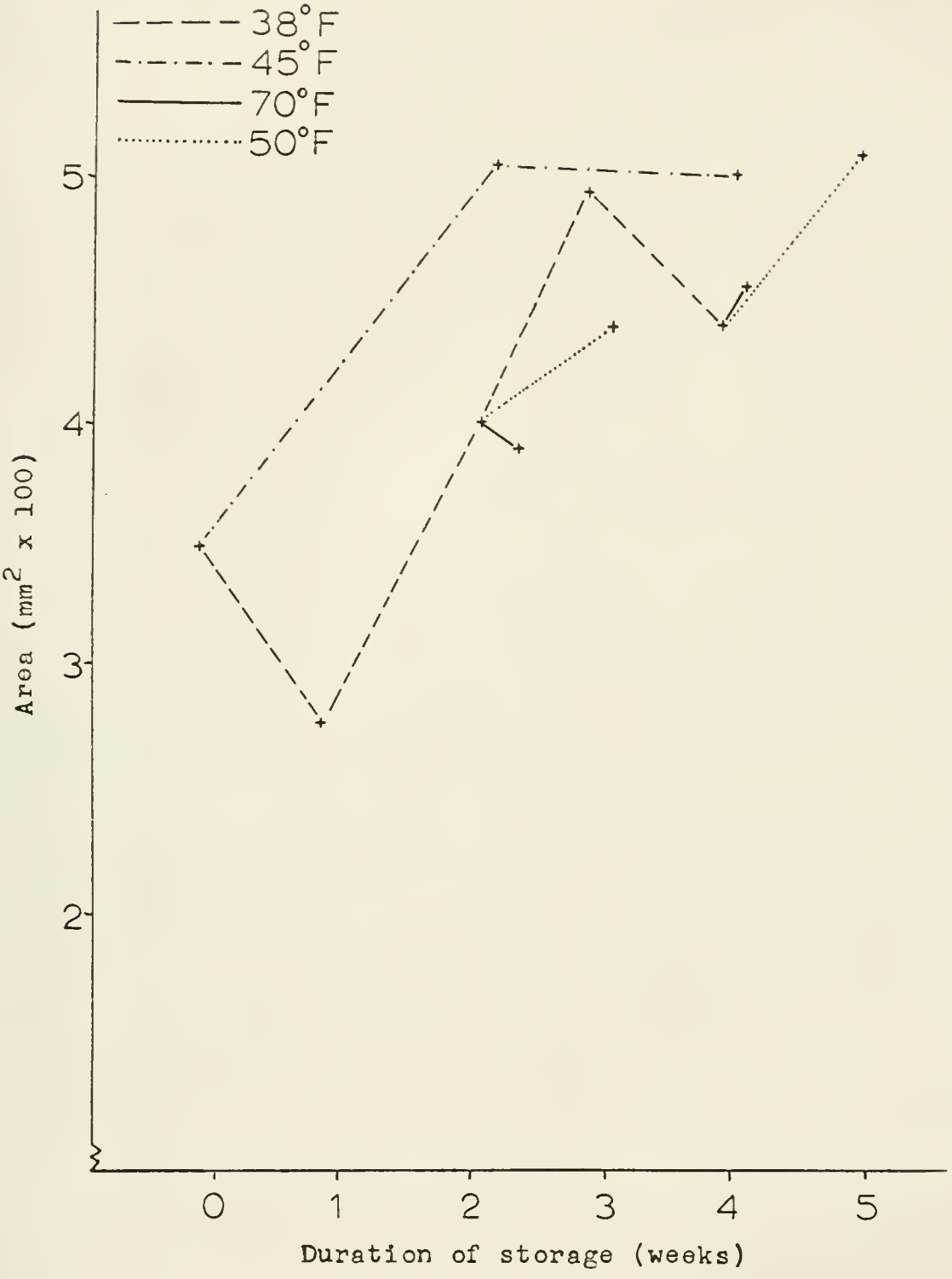


Figure 22. General trends in total peak area (dry weight basis) according to temperature and duration of storage at 45°F, 38°F, and subsequent storage at 50°F and 70°F after storage at 38°F.

there was an increase in volatile content from harvest to 2 weeks' storage whereas there was little change through 4 weeks' storage. In fact, all storage treatments except those at 70°F resulted in increases in measured volatile content at some time during storage. From these data it appears that there is a synthesis of the $C_{10}H_{16}$ hydrocarbons in storage at temperatures of 38°F, 45°F and 50°F. However, a loss of these hydrocarbons during storage at 70°F indicates they are metabolized or otherwise partially lost through a physical system. It is not possible to determine from these data what limiting factors caused a decrease in the buildup of these components after 2 weeks' storage of celery at 38°F and 45°F.

The reactions taking place in the holding flask have not been studied in these experiments; however, the temperature of the holding flask (55°C) is near the destructive point of most enzymes. If the enzymes controlling the production of $C_{10}H_{16}$ hydrocarbons were functional at this temperature, the amount of volatile components present after 2 hours' holding time would relate directly to the amount of substrate present in the tissue.

The biogenesis of terpenes is not fully understood; however, it does involve the condensation of isoprene units through mevalonic acid and squalene; mevalonic acid being formed from acetic acid in plant tissue (5). The mechanism involves condensation of acetyl CoA and acetoacetyl CoA to beta-hydroxy-beta-methyglutaryl CoA. The latter then is

reduced to form the mevalonic acid. Since the precursors of the terpenes are common metabolic agents, the synthesis or degradation of these products should be temperature dependent and subject to the same limitations of other metabolic activities. A buildup of the terpenes could only be explained as a result of the degradation of other metabolites (such as beta oxidation of fats) to form available acetyl CoA and acetoacetyl CoA. The amounts of available acetyl CoA and acetoacetyl CoA required to influence volatile changes are not known.

It is a known phenomenon that cutting of tissue results in an increase in metabolic activity. The injury to the tissue in these experiments was maintained constant and should not have affected the results.

The changes in moisture content did not greatly effect measured peak area in the head-space experiment. There was no significant correlation between celery dry weight and measured volatile content as based on correlation coefficients. The increase in dry weight of celery stored 2 or 4 weeks at 45°F was a result of inadequate control of humidity, even though celery was placed in plastic bags to minimize water loss. A larger buildup of the volatile constituents with these treatments could have been indirectly affected by the reduced moisture content.

The 9 volatile components measured in the head-space analyses correspond roughly to the chromatographic fraction of the solvent extracts with retention times of 8 to 20

minutes. There was a considerable decrease (Figure 7) in the quantity of these components being measured when celery was stored at 70°F for 5 days. Also, storage of celery for 2 weeks at 45°F and 1 additional week at 50°F resulted in an increase in these components and a highly significant increase in the amount of limonene (Figure 10). The changes in the $C_{10}H_{16}$ hydrocarbons in the head-space analyses corresponded to those observed when celery solvent extracts were prepared from celery stored under market conditions and at 70°F. Storage of celery for 2 weeks at 38°F resulted in a significant increase in the peak area of limonene (solvent extraction) while after 4 weeks' storage at 38°F the content of limonene (solvent extraction) was not different from the control (Table 6). While no significant difference was observed between the limonene content (head-space) of the control celery and samples stored 2 weeks at 38°F, the treatment at 38°F did result in a larger peak area and all 4 $C_{10}H_{16}$ hydrocarbons analyzed followed the same general trend.

The data substantiate the idea that storage of celery at 70°F results in a high ratio of high/low boiling components and presumably in a stronger (but not necessarily more desirable) celery flavor, while storage at temperatures between 38°F and 50°F result in lower ratios of high/low boiling components and a possible reduction in strong celery flavor. These assumptions should be confirmed through rigorous experiments using organoleptic procedures.

Component Distribution

Considerable quantitative difference was observed between the high and low boiling components in the chromatograms prepared from the various parts of the stalk. Table 14 presents the data for the ratio of high/low boiling components. The mean ratio for the top was significantly different from the middle and outer portions of the stalk, while the ratio for the outer portion was significantly higher than any other portion.

Table 14. Ratios of high/low boiling fractions from chromatograms of extracts prepared from various parts of the celery plant.

Top	Inner	Bottom	Middle	Outer
0.66*	0.88	1.09	1.23	1.80

*Those values not connected by a continuous line are significantly different (0.05 level) according to Duncan's multiple range test.

A qualitative difference was observed between the chromatograms from the top (leafy) portion and all other portions of the celery stalk. The chromatograms prepared from the top portion had a large peak at 5 minutes, 25 seconds when chromatographed on a column of Apiezon L, and 5 minutes, 15 seconds when chromatographed on Carbowax 20 M. This component occupied as much as 40 per cent of the total peak area of these chromatograms and was absorbed by a

column containing boric acid which indicated it was an alcohol (27). The odor of this compound was very ether-like when detected at the exhaust port of the gas chromatograph. Standard alcohols were chromatographed to aid in prediction of carbon number and are plotted against retention times in Figure 23.

The chromatogram from the top (leafy) extract is presented in Figure 24. In addition to the unknown alcohol previously mentioned, other qualitative variations in this sample occurred near the retention time of 72 minutes. In all other chromatograms the peak at 72 minutes appeared as a major peak with a small shoulder. However, observation of the chromatogram from the top (leafy) extract shows that two peaks are completely resolved and present in large quantities.

Chromatograms from celery extracts of inner and outer portions are presented in Figure 25. While both fractions showed a reduced amount of limonene as compared to other fractions, the samples extracted from the inner portion of the stalk had a larger quantity of the other components with retention times of 10 to 40 minutes, yielding a lower ratio of high/low boiling components. In addition to the comparatively low amount of low boiling fraction in the extracts of the outer portion, there was a larger amount of those components with retention times of 60 to 90 minutes; thus there was a higher ratio of high/low boiling components. While few qualitative differences can be seen between these

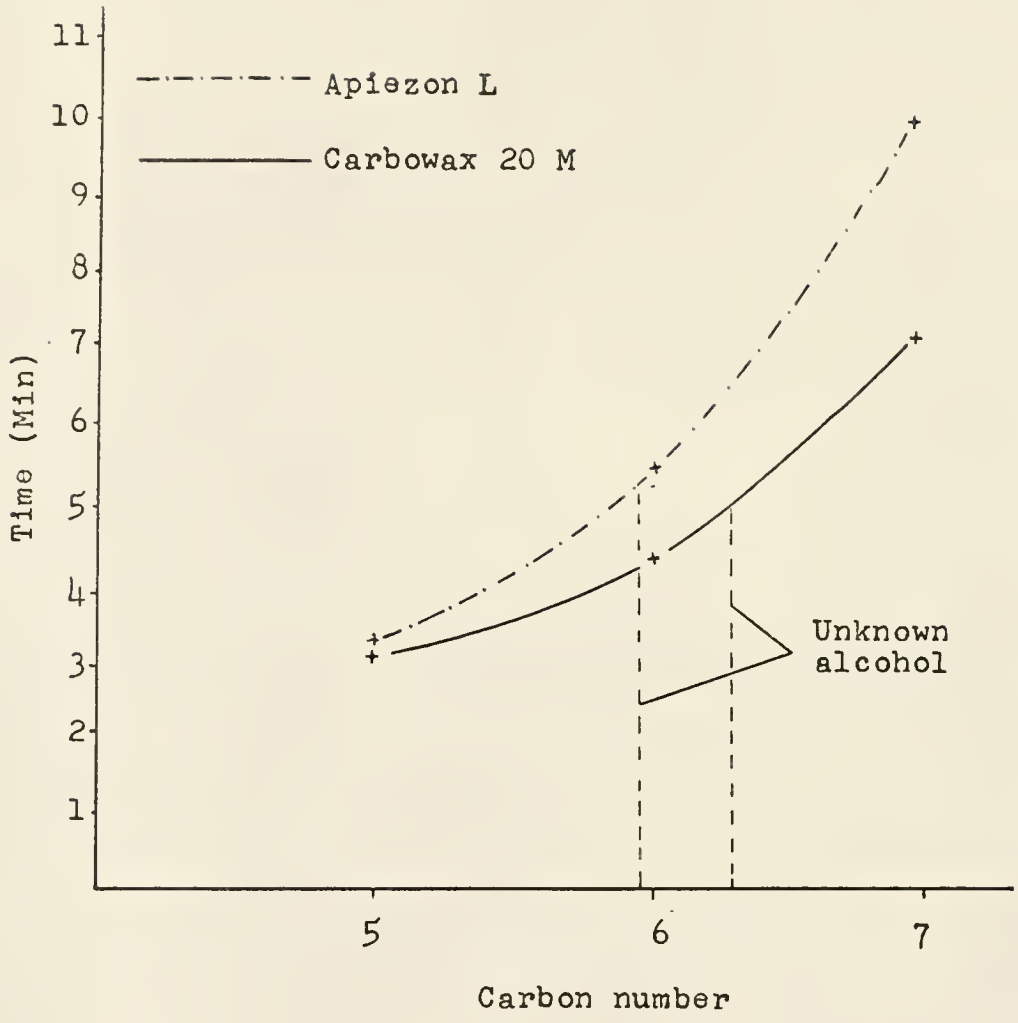


Figure 23. Retention time of 5, 6, and 7 carbon straight chain alcohols for polar and non-polar chromatographic phases.

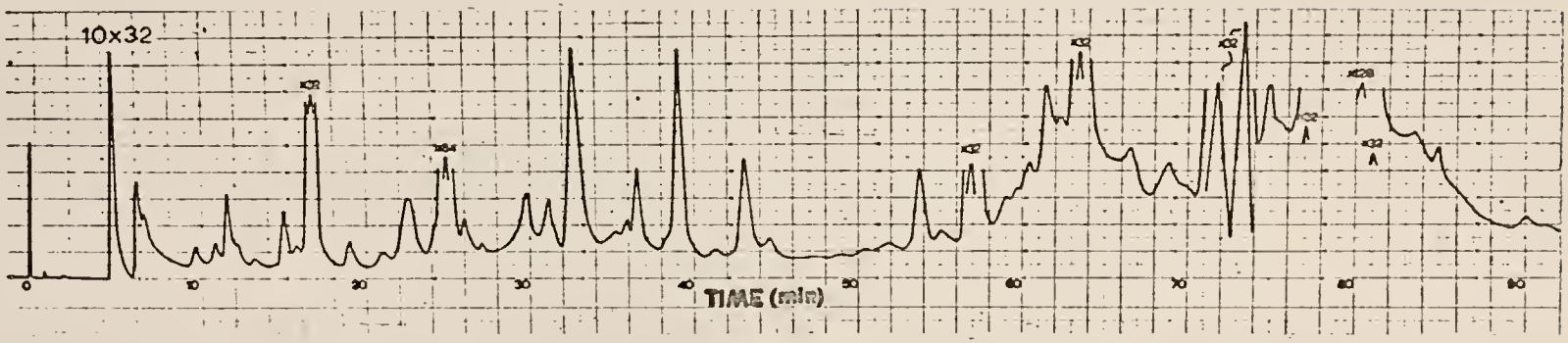


Figure 24. Chromatogram prepared from an extract of the top (leafy) portion of the celery plant.

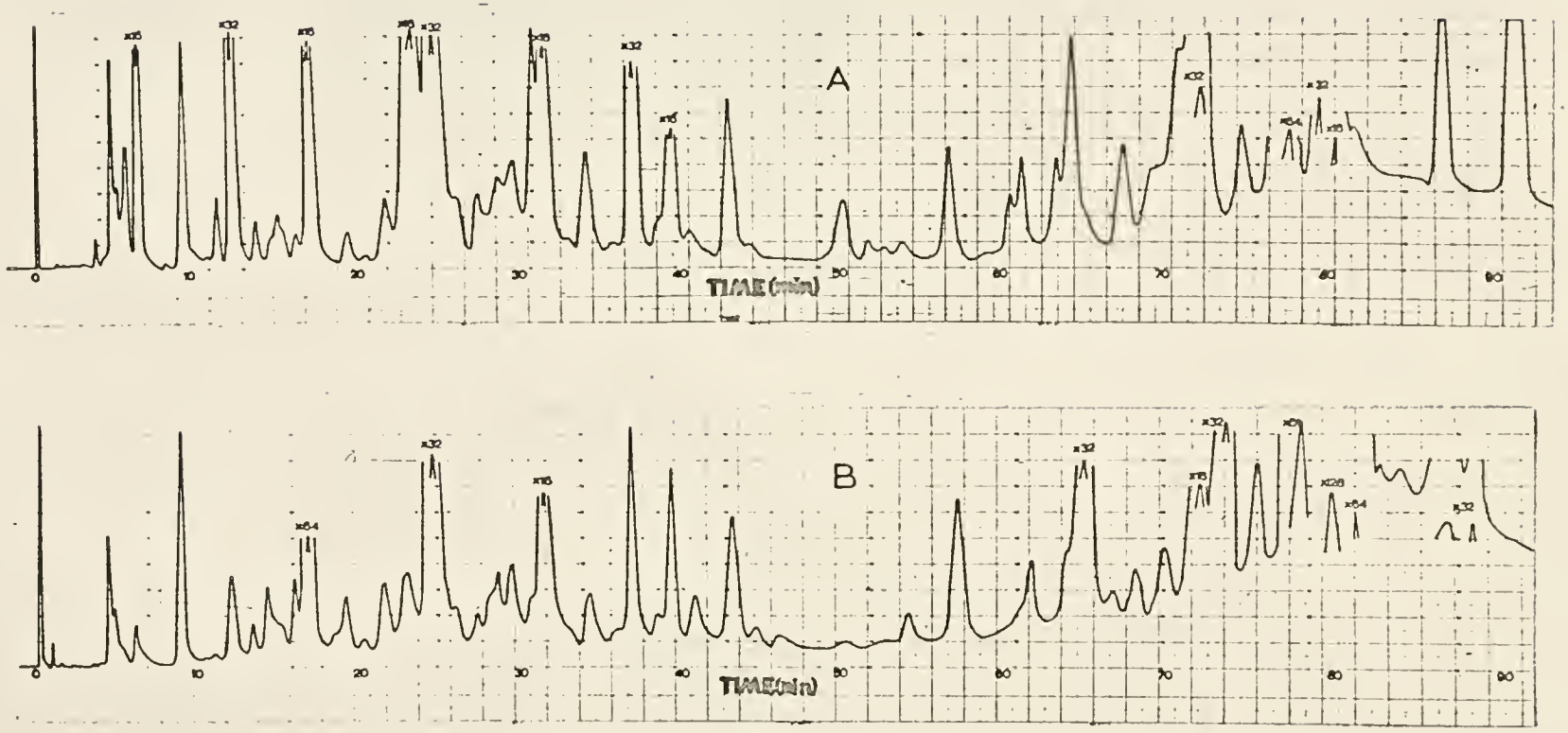


Figure 25. Chromatograms prepared from celery extracts of (A) the inner and (B) outer portion of the stalk.

chromatograms, the fact that the inner portion of the stalk resulted in a low, high/low ratio and the outer portion a high, high/low ratio substantiates the differences in ratio of high/low fractions previously mentioned.

Chromatograms from the middle and bottom portions of the stalk are comparable to those of the storage treatment extracts and little quantitative or qualitative difference was observed. These results should be expected since the celery used in the preparation of the storage treatment extracts did not contain leafy portions and was primarily composed of middle and bottom portions of the stalk.

While Pan (36, 37) and Hall (29) did not measure differences in the volatile constituents of celery, their research seems to substantiate these data. It was noted that the strong bitter celery flavor was associated with the outer petioles and with the more green portions of the stalk. From these data, it is not possible to determine if the high ratio of high/low boiling components is associated with a strong celery flavor. However, according to the odor characteristics presented in Table 3, this would appear true. Also, in accordance with these data, Hall (21) found organoleptic differences between middle and upper portions of the outer and inner petioles of the same stalk.

Taste panel comparisons have been made between juice expressed from the top (leafy) section of the celery plant and that expressed from the bottom portion below the leaves (14). The panel was able to differentiate between the two

samples at the 0.01 level of statistical significance. However, these investigators indicated it was not possible to differentiate between the two juices on the basis of the gas chromatograms of their distillates.

Leaf alcohol (hex-cis-3 enol) is widely distributed in green plants and is reported to be primarily responsible for the odor of foliage plants (40, 42). Since the alcohol in the celery extracts had a retention time similar to the 6 carbon group (Figure 23), it is possible that it is closely related to the above-mentioned leaf alcohol and not a specific product of celery. Noteworthy is the fact that no peak corresponding to this retention time could be detected when leafy portions of celery were analyzed using head-space procedures.

SUMMARY AND CONCLUSIONS

Odor detection of the various components in the celery extracts revealed that those components with retention times of 72 to 85 minutes possessed the characteristic odor of celery. Analysis of the various chromatograms revealed that two areas of high concentration were present and were classified into high and low boiling components. Further investigation of these areas revealed that net area changes in the chromatograms could be detected by use of the ratio of high/low boiling components.

Storage-induced flavor changes in celery stalks appear to be quite temperature dependent when analyzed by methods used in this research. Storage of celery at 70°F, whether fresh or previously stored at 38°F, resulted in a decrease in the amount of $C_{10}H_{16}$ hydrocarbons. Also, complete flavor profile analysis showed a highly significant decrease in total peak area of the chromatograms from celery stored 5 days at 70°F. While all components decreased when celery was stored under these conditions, the ratio of high to low boiling components increased significantly.

When celery was stored under market simulated conditions (2 weeks at 45°F and 1 subsequent week at 50°F) the ratio of high/low boiling components decreased

significantly. This decrease was probably due to increases in the peak area of the $C_{10}H_{16}$ hydrocarbons. When celery was stored at 38°F or 45°F, and analyzed using head-space techniques, the $C_{10}H_{16}$ hydrocarbons content was higher after 2 weeks' storage than for freshly harvested celery (FWB); no further increase was noted with longer storage at these temperatures. Storage at 50°F subsequent to storage at 38°F also resulted in increases in these components. Previous investigations (5) have established that the basic mechanism of synthesis for these components is the condensation of acetyl CoA and acetoacetyl CoA; however, it is not known what limitation might cause a reversal in the trend of component increase at 70°F storage.

Since other investigators have discussed the importance of the high boiling phthalides in celery flavor (10, 14, 15, 16, 20, 28, 32), the changes in the ratio of high/low boiling fractions seem to have importance. In view of these changes, it would appear that storage of celery at 70°F would result in a stronger celery flavor, while storage at 38°F, 45°F, or combinations of these temperatures with 50°F storage would result in celery with less intense celery flavor as compared to the same celery at harvest.

The same theory applies to the ratio of these components in various portions of the stalk and is supported by several investigators (14, 21, 22, 23). In general, the strong portion of the celery flavor is associated with the greener portions of the stalk, in particular the outer

petioles. This is further supported by the fact that the inner (heart) petioles had a ratio of high/low boiling fraction which was significantly lower than either the middle or outer portions.

Further research should establish the organoleptic association with these data. This would require rigorously controlled conditions since celery stalks have considerable changes in texture and appearance with long time storage.

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BIOGRAPHICAL SKETCH

Danny Odell Ezell, the son of Odell W. and Madge G. Ezell, was born June 3, 1941, at Spartanburg, South Carolina. In June, 1958, he was graduated from Chesnee High School, Chesnee, South Carolina. In June, 1962, he received the degree of Bachelor of Science with a major in Agricultural Education from Clemson University. After graduation, he enrolled in the Graduate School of Clemson University. He worked as a graduate assistant in the Department of Horticulture until June, 1964, when he received the degree of Master of Science. From September, 1964, until the present time he has pursued his work toward the degree of Doctor of Philosophy.

Danny Odell Ezell is married to the former Elwanda Dayle Henderson. He is a member of Alpha Tau Alpha, Alpha Zeta, Gamma Sigma Delta, and the American Society for Horticultural Sciences.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

June 1968

F. L. Thomas and

Dean, College of Agriculture

Dean, Graduate School

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